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MINIREVIEW

The chicken gastrointestinal microbiome

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Abstract

The domestic chicken is a common model organism for human biological research and of course also forms the basis of a global protein industry. Recent methodological advances have spurred the recognition of microbiomes as complex communities with important influences on the health and disease status of the host. In this minireview, we provide an overview of the current state of knowledge of the chicken gastrointestinal microbiome focusing on spatial and temporal variability, the presence and importance of human pathogens, the influence of the microbiota on the immune system, and the importance of the microbiome for poultry nutrition. Review and meta-analysis of public data showed cecal communities dominated by Firmicutes and Bacteroides at the phylum level, while at finer levels of taxonomic resolution, a phylogenetically diverse assemblage of microorganisms appears to have similar metabolic functions that provide important benefits to the host as inferred from metagenomic data. This observation of functional redundancy may have important implications for management of the microbiome. We foresee advances in strategies to improve gut health in commercial operations through management of the intestinal microbiota as an alternative to in-feed subtherapeutic antibiotics, improvements in pre- and probiotics, improved management of polymicrobial poultry diseases, and better control of human pathogens via colonization reduction or competitive exclusion strategies.

Introduction

We are in the midst of what may, in retrospect, come to be referred to as the golden age of microbial ecology. The microorganisms and their genes associated with higher organisms (the microbiome) that were once viewed primarily as sources of human pathogens are now recognized as complex communities with important influences on the health and disease status of the host. Indeed, it has been suggested that humans (and other multicellular organisms) should actually be considered as 'supra-organisms' interacting in concert with their microbiomes (Turnbaugh *et al.*, 2007).

The domestic chicken, *Gallus gallus domesticus*, with a global population exceeding 40 billion individuals per year (Muir *et al.*, 2008) has a unique status as 'both the model and the system' – chickens are common model organisms for human biological research and also comprise an economically valuable global protein industry. In this minireview, we synthesize material from previous studies of the poultry microbiome (Barnes, 1979; Zhu *et al.*, 2002; Lu *et al.*, 2003; Gabriel *et al.*, 2006; Lee & Newell, 2006; Qu *et al.*, 2008; Danzeisen *et al.*, 2011; Yeoman *et al.*, 2012; Oakley *et al.*, 2013; Zhao *et al.*, 2013) and focus on the gastrointestinal tract as the area with highest bacterial abundance and diversity (O'hara & Shanahan, 2006) and greatest relevance to animal health, nutrition, food safety, and public health. We briefly discuss the chicken microbiome from crop to cloaca and farm to fork, focusing on spatial and temporal variability,

the presence and importance of human pathogens, the influence of the microbiota on the immune system, and the importance of the microbiome for poultry nutrition.

Methods of study

Until recently, the view of the microbiome was restricted to those microorganisms that could be recovered on growth media. We now know that cultivation techniques which form the basis of classical microbiology do not recover the majority of microorganisms (Rappe & Giovannoni, 2003); for bacterial taxa inhabiting the poultry gastrointestinal tract, perhaps < 20% have been recovered by cultivation (Gaskins *et al.*, 2002). Within the last decade, several technical advances have allowed new insights into this uncultured majority. First, direct sequencing of 16S rRNA genes has provided a powerful method to profile complex microbial communities without relying on cultivation. Second, in tandem with 16S rRNA gene-based taxonomic census data, metagenomics has begun to fulfill early expectations of revolutionizing our understanding of microbial communities (NRC Committee on Metagenomics, 2007). Metagenomics (and related approaches, metatranscriptomics and metaproteomics) directly sequences genes (or transcripts, or proteins) present in a sample, independent of cultivation biases or PCR targeting a specific gene (Handelsman, 2004). By directly sequencing a sample, these meta-omics approaches have provided important insights into the metabolic functioning of bacterial communities (Tringe *et al.*, 2005; Frias-Lopez *et al.*, 2008; Qin *et al.*, 2010) that would not have been possible previously. Third, single-cell microbiology is a rapidly emerging and powerful set of approaches largely driven by the realization that populations of cells previously considered as clonal entities are in fact genotypically and phenotypically heterogeneous. This variability has important implications for physiology, evolution, ecology, and pathogenesis (Davey & Kell, 1996; Brehm-Stecher & Johnson, 2004). Single-cell microbiology has not yet achieved widespread adoption in the veterinary sciences, but already has demonstrated the potential for much more sophisticated queries than previously possible of the mechanisms underlying the ecology of complex microbial communities (Huang *et al.*, 2007; Marcy *et al.*, 2007; Li *et al.*, 2008; Musat *et al.*, 2008; Stecher *et al.*, 2013). We anticipate that single-cell approaches will provide important insights into the chicken microbiome in the near future.

Spatial variability

As feed passes through the gastrointestinal tract, it encounters specialized microbial communities that perform important digestive functions. Briefly, beginning in the crop, starch breakdown, and lactate fermentation are mediated by a community dominated by various *Lactobacillus* spp. at cell densities up to 10^9 g^{-1} (van der Wielen *et al.*, 2002; Rehman *et al.*, 2007; Stanley *et al.*, 2014). Lactobacilli also dominate the proventriculus, a thick-walled stomach, and the ventriculus (gizzard). The gizzard has been described as the 'teeth' of the gastrointestinal tract where a majority of mechanical and chemical breakdown of feed is performed (Ensminger, 1971; Rehman *et al.*, 2007); the low pH of gastric juices containing hydrochloric acid and pepsin limits the total number of cells below 10^8 g^{-1} (Yeoman *et al.*, 2012). The small intestine harbors large (10^9–10^{11} cells g^{-1}) bacterial populations dominated by *Lactobacillus*, *Enterococcus*, and various Clostridiaceae (van der Wielen *et al.*, 2002; Rehman *et al.*, 2007; Kohl, 2012; Pan & Yu, 2013; Stanley *et al.*, 2014; Waite & Taylor, 2014). Several excellent recent reviews provide additional details regarding the taxonomic composition of microbial communities typically found in the different sections of the gastrointestinal tract (van der Wielen *et al.*, 2002; Rehman *et al.*, 2007; Kohl, 2012; Yeoman *et al.*, 2012; Pan & Yu, 2013; Stanley *et al.*, 2014; Waite & Taylor, 2014). Here, we focus on the ceca as organs of particular interest as they harbor the highest microbial cell densities (up to 10^{11} cells g^{-1}), have the longest residence time (12–20 h) of digesta in the gastrointestinal tract, and are important sites for recycling of urea, water regulation, and carbohydrate fermentations contributing to intestinal health and nutrition (Ensminger, 1971; Clench & Mathias, 1995; Sergeant *et al.*, 2014; Waite & Taylor, 2014) as described in more detail below. Although chickens can survive with experimentally removed ceca (Clench & Mathias, 1995), around 10% of energy may be provided by digestive processes in the ceca where short chain fatty acids (SCFAs) concentrations are higher than elsewhere in the gastrointestinal tract (Józefiak *et al.*, 2004). Currently, the largest volume of sequence data exists for cecal microbial communities, facilitating direct comparisons across multiple studies, which is hindered by other data types such as T-RFLP or DGGE. Firmicutes, Bacteroides, and Proteobacteria are the most common phyla in the chicken ceca, with Actinobacteria accounting for the remainder (Fig. 1a). At finer scales of taxonomic resolutions, the majority of sequence types can be shown to belong to various members of the Clostridiales (Fig. 1b). Although Clostridiales are known generally as important contributors to SCFA metabolism, understanding in greater detail the functional niches of the members of this diverse group and their interactions remains an important topic for future study.

Although relatively few studies to date have examined the metabolic capabilities of the chicken cecal microbiome

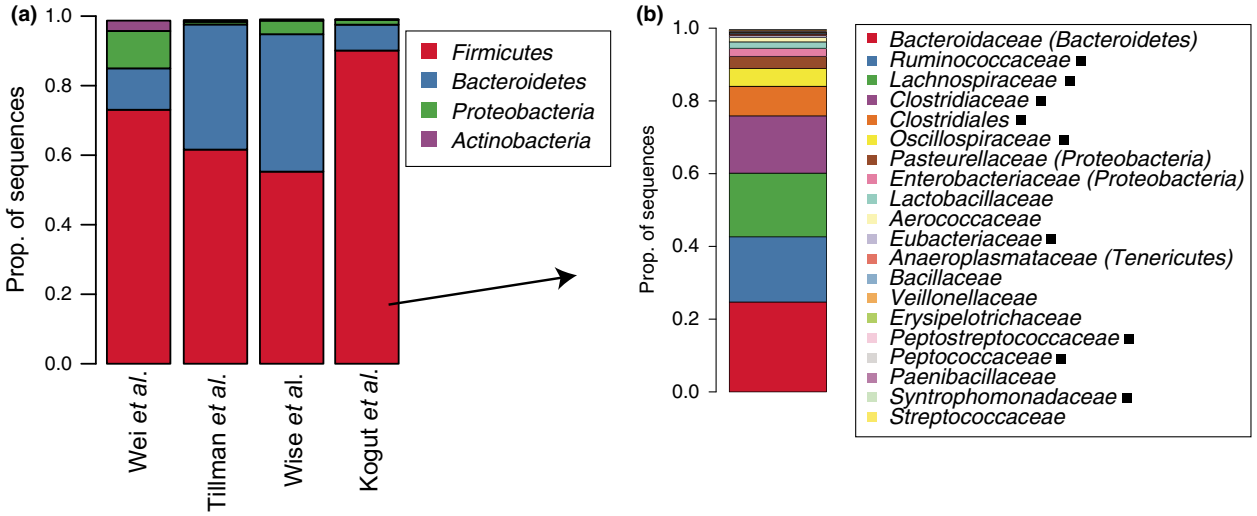

Fig. 1. Relative proportions of bacterial phyla (a) and families (b) found in chicken ceca. Data from Wei *et al.* (2013) represent publically available sequences retrieved as described. Data from Tillman *et al.* (2011) and Wise & Siragusa (2007) are re-analyzed from data included in (Oakley *et al.*, 2013) representing 8 and 10 birds, respectively. Kogut *et al.* data are unpublished, collected, and analyzed as previously described (Oakley *et al.*, 2012 b, 2013) representing 20 birds and *c.* 20 000 sequencing reads. Data for each of these three flocks are from 3 weeks posthatch. Sequences from Wei *et al.* were additionally screened by removing sequences with ambiguous base calls, and all sequences were classified against a reference database of type strains from SILVA v115 (Pruesse *et al.*, 2007). Many of the sequences reviewed in (Wei *et al.*, 2013) do not contain metadata regarding bird age, which can have strong effects on community composition and structure. For (b) families belong to the phylum Firmicutes unless otherwise noted; families followed by black squares belong to the Clostridiales.

via metagenomics, some valuable insights have already been obtained. As might be inferred from the taxonomic composition of the cecal microbiome, genes associated with carbohydrate metabolism are consistently abundantly represented in metagenomic libraries (Qu *et al.*, 2008; Danzeisen *et al.*, 2011; Sergeant *et al.*, 2014). Of particular interest is a recent study in which over 200 different nonstarch polysaccharide (NSP) degrading enzymes were found, including a potentially novel pathway for propionate production (Sergeant *et al.*, 2014). Additionally, the discovery of uptake hydrogenases from some of the most abundant genera (*Megamonas*, *Helicobacter*, and *Campylobacter*) supports interesting speculations regarding a possible mechanism by which SCFA production is promoted by these taxa acting as hydrogen sinks (Sergeant *et al.*, 2014).

One of the most important observations of the human microbiome project has been to demonstrate a conservation of metabolic function despite large taxonomic variability across individuals (Turnbaugh *et al.*, 2009). Similar patterns in chickens might suggest similar mechanisms governing community assembly, structure, function, and host selection. To test this, we performed a meta-analysis of public data from previous observations in chickens (Qu *et al.*, 2008; Danzeisen *et al.*, 2011) and found a very similar pattern to the human microbiome with community composition taxonomically variable, but functionally conserved among individuals (Fig. 2). This observation provides support for inferences made from human microbiome data to the chicken microbiome and has important implications for management of the microbiome as similar metabolic functions appear to be carried out by a phylogenetically diverse assemblage of microorganisms. In a metagenomic study of the chicken cecal microbiome, Qu *et al.* (2008) found that *c.* one-fourth of assembled contigs were most closely related to transposes, suggesting that lateral gene transfer might be an important mechanism contributing to a 'pan-genome' under selective pressure for functional traits shared by multiple taxa.

Successional development

In most commercial poultry operations in the United States, a diverse microbial community in the housing environment (mainly in the litter) is carried over from one flock to the next and thus can serve as an important inoculum for the chick gastrointestinal microbiome (Liljebjelke *et al.*, 2003). Newly hatched chicks coming from hatcheries have no contact with adult birds, and thus environmental microbial communities, of which the litter is likely the most important, function as important inocula that can shape the development of the gastrointestinal microbiome and potentially carry through the life of a

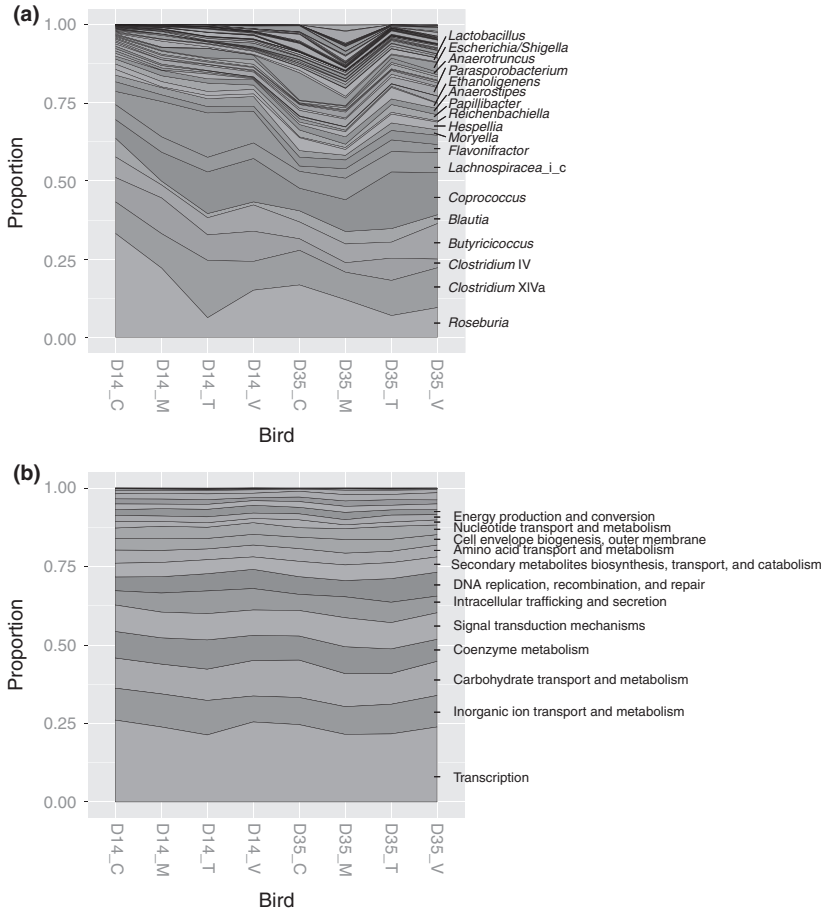

Fig. 2. Taxonomic and functional variability within the chicken cecal microbiome. Taxonomic classifications (a) and metabolic functional groupings (b) are re-analyzed from Danzeisen *et al.* (2011). Taxonomy is from the RDP 16S rRNA gene training set nine classified with the RDP classifier (Wang *et al.*, 2007); genera present at a mean relative abundance > 2.5% are labeled in (a). Functional classifications (b) are based on COG classifications by local RPS-BLAST against the February 2014 version of the conserved domain database.

flock. This possibility is supported by re-analysis of data from a recent paper (Oakley *et al.*, 2013) to show *c.* 50 bacterial genera common to litter, fecal, and carcass samples from two flocks followed longitudinally from 'farm to fork' (Table 1). The presence of several putative pathogens (Table 1) may have important implications for this management practice (Pedroso *et al.*, 2013).

Through the life span of a commercial broiler (typically 42 days), significant changes in the taxonomic composition of the gastrointestinal microbiome have been observed (Lu *et al.*, 2003) and are well reviewed elsewhere (Rehman *et al.*, 2007; Yeoman *et al.*, 2012; Stanley *et al.*, 2014). Analysis of our own previously unpublished data indicates clear successional changes in the taxonomic composition of the cecal microbiome can be observed during the life cycle of commercial broilers significantly associated with time and commonly used changes in diet [Fig. 3a and (Lu *et al.*, 2003)]. Interestingly, variability is much higher for fecal vs. cecal samples (Fig. 3b vs. 3a), supporting previous suggestions (Stanley *et al.*, 2014) that fecal samples may not be properly representative of the gastrointestinal tract due to differential mixing effects and the less frequent voiding of the ceca compared to the rest of the gastrointestinal tract. How successional changes in taxonomic composition relate to changes in metabolic functioning and morphological development of the intestine remains an important gap in our current knowledge of the chicken gastrointestinal microbiome. When available, such meta-omic data will provide important mechanistic insights into how the microbiome contributes to host development and nutrition.

Roles and importance of human and chicken pathogens

The chicken intestinal microbiome commonly contains several taxa capable of causing significant illnesses in humans, most importantly *Campylobacter* and *Salmonella*. *Campylobacter* spp. (mostly *C. jejuni* and *C. coli*) are present in nearly all birds at up to 10^7 CFU g^{-1} in the chicken intestine (Stern *et al.*, 1995) and cultivable by week three from the poultry environment (Lee & Newell, 2006). *Campylobacter* is generally accepted to be nonpathogenic in its avian host (Lee & Newell, 2006). *Salmonella* is a minor taxon in the chicken intestinal microbiome, sporadic in its distribution in poultry

Table 1. Genera common to fecal, litter, and carcass samples

Fusobacteria/Clostridium XIX	*Subdoligranulum*
Bacteroides	*Ruminococcus*
Sporacetigenium/Clostridium XI	*Roseburia*
Corynebacterium	*Butyricicoccus*
Peptostreptococcus/Clostridium XI	*Alkalibaculum*
Lactobacillus	*Eubacterium*
Brevibacterium	*Bacillus*
Faecalibacterium	*Butyricimonas*
Pseudoflavonifractor/Oscillibacter	*Papillibacter*
Clostridium	*Gallibacterium*
Oscillibacter	*Acetanaerobacterium*
Helicobacter	*Caloramator*
Salinicoccus	*Coprobacillus/Clostridium* XVIII
Campylobacter	*Escherichia/Enterobacter*
Brachybacterium	*Shigella/Escherichia*
Alkaliphilus/Clostridium XI	*Crinalium*
Enterococcus	*Paenibacillus*
Staphylococcus	*Veillonella*
Brevundimonas	*Leuconostoc*
Nosocomiicoccus	*Planktothrix*
Parabacteroides	*Citrobacter/Escherichia/Shigella*
Flavonifractor	*Weissella*
Tepidibacter	*Pseudomonas*
Anaerotruncus	*Massilia/Naxibacter*
Fusobacterium/Clostridium XIX	*Cloacibacillus*
Coprococcus	
Phascolarctobacterium	

Classifications are from best matches using global usearch against a reference database of type strains from v115 of the SILVA project. Taxonomy from the RDP 16S rRNA gene training set nine using the RDP naïve Bayesian classifier is shown after the backslash when different from the SILVA classification. Data re-analyzed from Oakley *et al.* (2013).

(Liljebjelke *et al.*, 2005) and transient in its colonization of its avian host (Gustafson & Kobland, 1984). *Salmonella* is capable of causing disease in avian species, but susceptibility to disease is dependent on age (Smith & Tucker, 1980), immune status (Phillips & Opitz, 1995), and *Salmonella* serovar or strain type (Jones, 1913; Barrow, 1991; Hernandez *et al.*, 2012).

Escherichia coli is another γ-proteobacteria present in the chicken intestine at low abundance throughout the life of the animal. Some *E. coli* strains are capable of causing opportunistic secondary infections in birds following other respiratory tract pathogens such as infectious bronchitis virus or *Mycoplasma gallisepticum* (Smith *et al.*, 1985; Gross, 1990) in response to high ammonia levels in poultry houses, or physiological changes in its avian host such as egg peritonitis or salpingitis in response to egg laying (Landman *et al.*, 2013). Unlike *E. coli* pathotypes that cause disease in other animal species, there is no clear set of virulence genes possessed by avian pathogenic *E. coli* (APEC) (Schouler *et al.*, 2012). Some APEC isolates from chickens may possess P-pili, S-pili, CNF toxin, Ibe proteins, or K1 capsule, which are virulence characteristics common to human extra-intestinal *E. coli* pathotypes; however these characteristics are found sporadically among avian isolates (Moulin-Schouleur *et al.*, 2006; Homeier *et al.*, 2010; Dziva *et al.*, 2013). APEC isolates that possess these virulence genes appear to be phylogenetically related to human extra-intestinal *E. coli* isolates that belong to the ECOR B2 group (Clermont *et al.*, 2000; Ewers *et al.*, 2007) and suggest some APECs could be zoonotic pathogens. However, these APEC strains, are generally nonhemolytic, do not possess the RTX hemolysin (*hlyA*) associated with human extra-intestinal *E. coli* pathotypes (Reingold *et al.*, 1999; Morales *et al.*, 2004; Piatti *et al.*, 2008; Wang *et al.*, 2009), and phylogenetic relationships based on signature chromosomal sequences do not hold up to whole genome sequencing (Johnson *et al.*, 2007; Dziva *et al.*, 2013). While APEC's potential as a zoonotic pathogen is uncertain (Dziva *et al.*, 2013), there is evidence that the

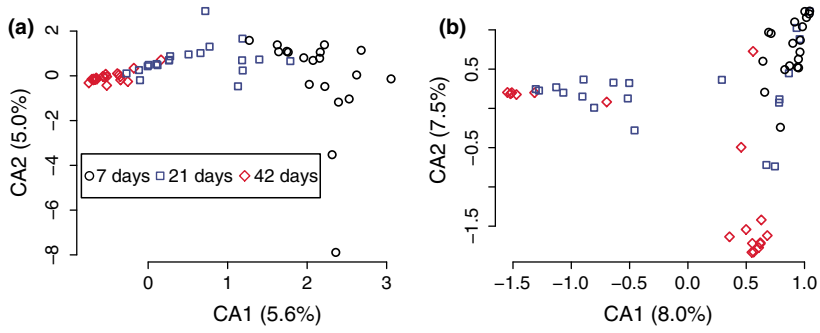

Fig. 3. Successional changes in the gastrointestinal microbiome in cecal samples (a) and fecal samples (b) associated with time and changes in diet as determined by principal components analysis of OTU classifications at a 3% dissimilarity cutoff. Data were analyzed in R with the VEGAN package. Each data point represents a single bird collected and analyzed as previously described (Oakley *et al.*, 2012a, b, 2013). For both cecal and fecal samples, clustering of the communities at each time point was significantly ($P < 0.001$) different as determined by permutational MANOVA. At 7 days, 'starter' feed contains c. 21% protein, at 21 days 'grower' feed 20%, and at 42 days 'finisher' feed contains c. 18% protein.

intestinal microbiome, including *E. coli*, may serve as reservoirs for antibiotic resistance and spread of resistance to zoonotic pathogens such as *Salmonella* (Nandi *et al.*, 2004; Fricke *et al.*, 2009).

The clostridial population includes some species pathogenic for poultry such as *Clostridium perfringens*, *C. septicum,* and *C. colinum* (Calnek, 1997). *C. perfringens* causes necrotic enteritis in poultry and isolates pathogenic for poultry contain a novel toxin type that is not possessed by human pathotypes (Keyburn *et al.*, 2010). Antibiotic feed supplements such as avoparcin, bacitracin, or virginiamycin are often used to prevent necrotic enteritis, and these antibiotics significantly affect the community structure of the intestinal microbiome (Lee *et al.*, 2006; Lu *et al.*, 2006; Lee, 2008).

Disease states without a clear etiologic agent are also found in poultry. For example, diagnoses of dysbacteriosis refer to undefined shifts in the intestinal microbiota associated with visible changes in the thickness, appearance, muscle tone, and tensile strength of the intestinal wall. Increased paracellular permeability enhances toxin and antigen penetration which stimulates inflammation, and the resulting changes in mucus production or composition attracts mucolytic species, such as *Clostridium perfringens,* that produce tissue damaging cytotoxins (Collier *et al.*, 2003, 2008). Similar polymicrobial syndromes have been observed in other hosts (Oakley *et al.*, 2008; Calvo-Bado *et al.*, 2011) and will most likely require a systems biology approach extending beyond a focus on specific pathogens.

Interactions with host immune system

Microbiota and the immune system

As an essential organ of the host mucosal immune system, the gut has evolved to carry out two apparently confounding tasks: nutrient absorption and pathogen defense. The intestinal immune system includes a robust mucosal layer, tightly interconnected intestinal epithelial cells (IEC), secreted soluble immunoglobulin A (IgA), and antimicrobial peptides (AMPs). It is well established that a beneficial microbial community has an important role in maintaining normal physiological homeostasis, modulating host immune system, and influencing organ development and host metabolism (Sommer & Backhed, 2013).

In poultry, there are few reports to date describing interactions between the gut microbiota and immune response. Forder *et al.* (2007) described a differential mucin profile and a greater numbers of goblet cells in the intestine of conventionally reared broiler chicks relative to isolator-reared broiler chicks and concluded these differences were due to differences in the microbiomes of the two groups. Similarly, differences in intestinal lymphocyte cell numbers and lymphoid cellular subsets have been reported in germ-free chickens compared to conventional chickens (Honjo *et al.*, 1993). Further, the diversity of the avian gut microbiota has been shown to affect the complexity of the T-cell receptor repertoire in both the gut and the spleen (Mwangi *et al.*, 2010). Because of the relative paucity of data for poultry, the following discussion highlights literature from other animal models which is relevant to the poultry microbiome-immune system link.

In addition to guiding the production of cytokines and chemokines and influencing the T-cell repertoire of the intestine, the gut microbiota also modulates B-cell response and IgA production. IgA secreted into the lumen plays an important role in pathogen binding and removal (Macpherson & Uhr, 2004), and microbial modulation of IgA homeostasis is, in part, dependent on the host protein programmed cell death 1 (PD1) expressed on T follicular helper cells in the germinal center (Kawamoto *et al.*, 2012). PD1-deficient mice with altered IgA repertoire showed altered gut microbiota composition with reduced *Bifidobacterium* and *Bacteroides* and increased Enterobacteriaceae (Kawamoto *et al.*, 2012). The gut microbiota also regulate the production of AMPs in the IECs that include defensins such as C-type lectins, ribonucleases, angiopoietin 4 and S100 proteins which rapidly kill or inactivate microorganisms (Gallo & Hooper, 2012). Some AMPs, such as α-defensins and β-defensin 1, are expressed constitutively (Putsep *et al.*, 2000), whereas others are microbially induced (Hooper *et al.*, 2003; Cash *et al.*, 2006).

Gut immune homeostasis is maintained by a complex network of cells and their secreted soluble products (Kamada *et al.*, 2013). Gut-resident phagocytes, for example, regulate unresponsiveness of the innate immune system to microbial ligands and commensal bacteria by limited level of pro-inflammatory molecule secretion upon stimulation (Kamada *et al.*, 2005; Denning *et al.*, 2007; Franchi *et al.*, 2012).

Pathogen exclusion strategies – past, present, and future

Over 40 years ago, Nurmi and Rantala introduced the term competitive exclusion (CE) describing reduced *Salmonella* colonization in birds orally gavaged as newly hatched chicks with intestinal contents from salmonellae-free birds (Nurmi & Rantala, 1973). CE generally refers to a reduction in colonization by a pathogen due to several possible mechanisms: physical occupation of a site, resource competition in a physical or chemical niche, or

direct physical or chemical insult to the potential colonist. Early observations that very low levels of *Salmonella* can colonize the intestinal tract of young broiler chicks, while older birds are resistant to infection led to the transfer of microbial communities from adults into day-old chicks which increased the resistance to salmonellae colonization as predicted (Pivnick & Nurmi, 1982; Cox *et al.*, 1990). Similar introductions of complex microbial communities have been used to successfully treat gastrointestinal disorders including recurrent *C. difficile* infections in humans (Anderson *et al.*, 2012; Lawley *et al.*, 2012; Van Nood *et al.*, 2013), although the underlying mechanisms remain poorly understood in all cases.

Despite this lack of mechanistic understanding, CE remains the most effective approach to prevent the intestinal colonization of live poultry by salmonellae and a variety of commercial products have been developed. The majority of reported research shows that undefined mixtures from the intestinal tract of healthy adult birds are more effective in preventing *Salmonella* colonization than the defined mixtures that have been marketed. As an alternative, used poultry litter from a flock exhibiting good performance and intestinal health or fresh litter inoculated with CE cultures can also be used to colonize newly hatched chicks (Schefferle, 1965; Lovett *et al.*, 1971; Cruz *et al.*, 2013). As more mechanisms of pathogen exclusion become understood, new approaches will become possible. For example, blocking the attachment mechanism of unfavorable organisms with a type-1 fimbria blocker can reduce their capacity to compete with the favorable organisms in the gut. Products that mimic docking sites for specific gut epithelia glycoproteins may be useful in preventing attachment and colonization by gut pathogens recognizing these sites (Spring, 1996; Finucane *et al.*, 1999; Giron *et al.*, 2002).

Importance of the gastrointestinal microbiome for gut health and nutrition

Poultry represents the most efficient form of terrestrial animal protein: a modern commercial chicken can gain 3.48 kg in body weight by consuming just 6.37 kg of feed in 49 days (Walk *et al.*, 2013). Although much of this efficiency is due to selective breeding and management practices such as supplementation of feed with exogenous enzymes, the importance of the gastrointestinal microbiome for poultry nutrition is increasingly being recognized. Gastrointestinal microorganisms can have negative effects on the host such as overstimulation of the immune system, enzymatic digestion of intestinal mucus, breakdown of bile, or production of harmful amino acid catabolites (Gaskins *et al.*, 2002), but a 'healthy' microbiota is considered a net benefit to the chicken. For example, gastrointestinal microbial communities have been shown to exclude pathogenic taxa (Nurmi *et al.*, 1992), promote beneficial development of the intestinal mucus layer, epithelial monolayer, and lamina propria (McCracken & Gaskins, 1999; Shakouri *et al.*, 2009), break down polysaccharides (Beckmann *et al.*, 2006; Qu *et al.*, 2008), and provide energy as amino acids and SCFA (van der Wielen *et al.*, 2000; Dunkley *et al.*, 2007). SCFA are important nutrients for the host and are known to stimulate increases in absorptive surface area (Dibner & Richards, 2005). SCFA also reduce colonic pH, which can inhibit bile catabolism and subsequent conversion to secondary bile acids (Christl *et al.*, 1997).

In modern poultry production, although diets typically meet and sometimes exceed vitamin requirements (Skinner *et al.*, 1992), the intestinal microbiota can also act as a complementary exogenous source. Members of the gut microbiota are able to synthesize vitamin K as well as most of the water-soluble B vitamins, such as biotin, cobalamin, folates, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, and thiamine (Ichihashi *et al.*, 1992).

Prebiotics

Several studies have shown that growth performance, feed efficiency, and gut health in broiler chickens can be improved by dietary prebiotics, nondigestible carbohydrates selectively stimulating the growth of beneficial bacteria (Xu *et al.*, 2003; Yusrizal & Chen, 2003; Yang *et al.*, 2008a b). For example, feed supplementation with 0.4% fructo-oligosaccharides (FOS) in broiler chickens significantly increased body weight gain, feed efficiency, the activities of protease and amylase, ileal villus height, and the growth of *Bifidobacterium* and *Lactobacillus* (Xu *et al.*, 2003). Similarly, mannan-oligosaccharides (MOS) supplementation in broiler diets has repeatedly been shown to significantly improve energy, protein, fiber, and carbohydrate digestibility and utilization (Kumprecht & Zobac, 1997; Samarasinghe *et al.*, 2003; Yang *et al.*, 2008a, b).

Probiotics

Probiotics appear to be most effective during the initial development of the microbiota, or after any dietary change or stress and following antibiotic therapy and thus can be interpreted in the context of the ecological phenomena of primary and secondary succession in which a community is established or re-established following a disturbance. Several studies have demonstrated that supplementing feed with probiotics containing *Lactobacillus* cultures can enhance body weight gain and feed efficiency and reduce mortality rate in broilers (Zulkifli *et al.*, 2000; Kalavathy *et al.*, 2003; Timmerman *et al.*, 2006). For

example, a mixture of 12 *Lactobacillus* strains reduced abdominal fat deposition, serum total cholesterol, low-density lipoprotein cholesterol, and triglycerides in broilers (Jin *et al.*, 1998).

In mice and humans, Firmicutes have been shown to have a positive relationship with the ability to harvest energy from the diet (Turnbaugh *et al.*, 2006, 2008; Jumpertz *et al.*, 2011), and the Firmicutes : Bacteroides ratio may also be important for optimum physiology and nutrition (Mariat *et al.*, 2009; De Filippo *et al.*, 2010; Bervoets *et al.*, 2013). An increase in fecal Firmicutes was associated with an increase in nutrient absorption, whereas an increase in fecal Bacteroidetes was associated with a decrease in nutrient absorption (Jumpertz *et al.*, 2011). In a search for probiotic strains related to chicken performance, Torok *et al.* (2011) identified sequences related to *Lactobacillus salivarius*, *L. aviarius*, *L. crispatus*, *Faecalibacterium prausnitzii*, *E. coli*, *Gallibacterium anatis*, *Clostridium lactatifermentans*, *Ruminococcus torques*, *Bacteroides vulgatus*, and *Alistipes finegoldii* from ileal and cecal samples. In the lowest portion of the small intestine, *Lactobacillus* spp. have been implicated as a causal factor in low performance (DeLange & Wijtten, 2010), suggesting the location of colonization by probiotic strains may affect performance. As methodological advancements continue, we envision continued progress toward the development of novel probiotic approaches.

Summary

The gut represents a complex microbial ecosystem consisting of trillions of commensal bacteria living in symbiosis with the host. For chickens, interactions between the host and the gastrointestinal microbiome play a crucial role in host physiological development, health, nutrition, and food safety. As both 'the model and the system', the chicken microbiome offers important opportunities for both basic and applied research. As new tools continue to be applied to the chicken gastrointestinal microbiome, important progress will likely be made in several areas of poultry management. In particular, we foresee advances in strategies to improve gut health in commercial operations through management of the intestinal microbiota as an alternative to in-feed subtherapeutic antibiotics, improvements in pre- and probiotics, improved management of polymicrobial poultry diseases, and better control of human pathogens via colonization reduction or competitive exclusion strategies.

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