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Prebiotics and gut microbiota in chickens

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One sentence summary: This article visits the current knowledge of the chicken gastrointestinal microbiota and reviews most recent publications related to the roles played by prebiotics in modulation of the gut microbiota.

ABSTRACT

Prebiotics are non-digestible feed ingredients that are metabolized by specific members of intestinal microbiota and provide health benefits for the host. Fermentable oligosaccharides are best known prebiotics that have received increasing attention in poultry production. They act through diverse mechanisms, such as providing nutrients, preventing pathogen adhesion to host cells, interacting with host immune systems and affecting gut morphological structure, all presumably through modulation of intestinal microbiota. Currently, fructooligosaccharides, inulin and mannanoligosaccharides have shown promising results while other prebiotic candidates such as xylooligosaccharides are still at an early development stage. Despite a growing body of evidence reporting health benefits of prebiotics in chickens, very limited studies have been conducted to directly link health improvements to prebiotic-dependent changes in the gut microbiota. This article visits the current knowledge of the chicken gastrointestinal microbiota and reviews most recent publications related to the roles played by prebiotics in modulation of the gut microbiota and immune functions. Progress in this field will help us better understand how the gut microbiota contributes to poultry health and productivity, and support the development of new prebiotic products as an alternative to in-feed antibiotics.

Keywords: prebiotic; oligosaccharides; gut microbiota; poultry

INTRODUCTION

The significant role of chicken gastrointestinal (GI) microbiota in health, productivity and disease has been well recognized (Oakley et al. 2014; Stanley et al. 2014a). Over the past decades, much effort has gone into optimizing the gut microbiota of chickens using dietary interventions. Among them, use of antibiotics at subtherapeutic levels has been the most popular and probably most effective strategy to enhance feed efficiency and to keep animals healthy. However, such a practice has been heavily criticized due to emergence of antibiotic resistance and its potential spread to human pathogens (Marshall and Levy 2011). One of the alternatives for antibiotic growth promoters that are receiving much attention is dietary fibers with prebiotic functions. A prebiotic is defined as ‘a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health’ (Roberfroid et al. 2010). This definition was recently refined to shift the focus from selective targets to microbial ecological functions within the gut. The new definition of a prebiotic is ‘a nondigestible compound that, through its metabolism by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host’ (Bindels et al. 2015).

While effects of prebiotics on poultry production parameters have been well reviewed (Patterson and Burkholder 2003; Barry et al. 2009; Gaggia, Mattarelli and Biavati 2010), much less has been documented concerning their impact on the gut microbiota and host immunity. Recent advances in high-throughput sequencing technologies have provided a more in-depth insight into bacterial diversity and allowed the study of microbiota–immune system interactions. This review aims to summarize the most recent researches (2007–15) on prebiotic effects in chickens with a specific focus on intestinal microbiota. First,
we present an overview on diverse bacterial communities of the chicken GI tract and their roles in maintaining intestinal health. Then, we review published studies on how individual prebiotics affected intestinal microbiota and propose additional studies to advance our understanding of the prebiotic mechanisms of action in chickens.

**THE CHICKEN GI TRACT MICROBIOTA**

The chicken GI tract harbors a very complex microbiota, with over 600 different bacterial species from more than 100 bacterial genera (Torok et al. 2011). In general, the most abundant phylum in the chicken intestinal microbiota is Firmicutes followed by two minor phyla, Proteobacteria and Bacteroidetes (Fig. 1). In addition, members of phyla Actinobacteria, Tenericutes (Waite and Taylor 2014), Cyanobacteria and Fusobacteria (Qu et al. 2008) can be found in very low abundance. Bacterial communities vary considerably by locations along the GI tract of chickens. Crop, gizzard and duodenum share similar microbiota, dominated by the genus Lactobacillus, as high as 99% in some birds (Gong et al. 2007; Sekelja et al. 2012). The highest diversity of Lactobacillus was observed in the crop (Gong et al. 2007). The jejunum is also dominated by Lactobacillus species, mainly L. salivarius and L. avius (Gong et al. 2007; Feng et al. 2010). The microbial composition of the ileum is more diverse and less stable compared with the duodenum and the jejunum. The ileum is dominated by Lactobacillus, Candidatus Arthromitus, Enterococcus, Escherichia coli/Shigella and Clostridium XI (Asrore et al. 2015; Pourabedin, Guan and Zhao 2015). The cecum is by far the most densely colonized microbial habitat in chickens and its bacterial diversity is much higher than those in the upper GI tract. The most detailed information regarding chicken gut microbiota is available for the cecum. The cecum is a key region for bacterial fermentation of non-digestible carbohydrate and a main site for colonization by pathogens. Chickens have two paired ceca, both harboring similar bacterial communities (Stanley et al. 2015). In a study by Gong et al. (2007), the cecum was mainly occupied by the Clostridia genus followed by genera Lactobacillus and Ruminococcus. The majority of Clostridium detected in the cecum fall primarily into three main families, Clostridiaceae, Lachnospiraceae and Ruminococcaceae (Danzeisen et al. 2011). Enterococcaceae, Enterobacteriaceae and Bacteroidiaceae are other reported abundant families in the cecal microbiota (Yin et al. 2010). The cecum is also rich in unknown and unclassified bacterial residents (Stanley et al. 2013a). At the species level, Bacteroides fragilis, L. crispatus, L. johnsonii, L. salivarius and L. reuteri comprise more than 40% of cecal microbiota (Stanley et al. 2015). To study GI microbiota, fecal samples are often used because of easy sampling. The composition of fecal microbiota highly fluctuates depending on varying contributions of microbiota from different GI segments (Sekelja et al. 2012). Lactobacillaceae, Peptostreptococcaceae, Streptococcaceae, Clostridiaceae and Enterobacteriaceae were identified as common families of the fecal microbiota (Videnska et al. 2014a). The fecal microbiota of laying hens is generally more complex than the fecal microbiota of broilers (Videnska et al. 2014a). Recently, Stanley et al. (2015) indicated that about 88% of all operational taxonomic units, comprising 99.25% of sequences, were shared between cecal and fecal samples in broiler chickens.

The GI microbiota of chickens could be separated into four potential robust clusters, referred to as enterotypes (Kaakoush et al. 2014), similar to the presence of three enterotypes in human gut microbiome (Arumugam et al. 2011). Enterotypes are in fact distinct bacterial communities, each dominated by different bacterial genera. Enterotypes in humans are correlated with long-term dietary patterns but independent of host phenotypes such as gender, age or body mass index (Wu et al. 2011).

![Figure 1. The chicken gut microbiome. The graphs provide an overview of the relative abundance of dominant bacterial phyla and families of the broiler chicken ileal (top level) and cecal (bottom level) microbiota in two different ages, 7 and 35 days. Data are compiled from three studies: Asrore et al. (2015) for ileum on day 7, Corrigan et al. (2015) for cecum on day 7 and Pourabedin et al. (2015) for ileum and cecum on day 35.](image-url)
However, whether such associations exist in chickens has not been studied. Despite existence of such enterotypes, there is a strong individual variation among chickens of a same breed, on a same diet and even under highly controlled experimental conditions (Nordentoft et al. 2011; Sekelja et al. 2012; Stanley et al. 2013b). This variation could be due to the fact that in the modern industrial poultry production, chickens are being hatched in highly hygiene incubators and reared without exposure to maternally derived bacteria. The random colonization by surrounding environmental bacteria is assumed to be a key reason for a high variation in the intestinal microbiota (Stanley et al. 2013b).

The chicken gut microbiota has been found to be affected by diet (Torok et al. 2008), gender (Lumpkins et al. 2008), background genotype (Zhao et al. 2013), housing condition (Nordentoft et al. 2011), floor litter (Torok et al. 2009; Cressman et al. 2010), feed restriction (Callaway et al. 2009) and stocking density (Guardia et al. 2011). Furthermore, as a bird ages, the microbiome complexity increases (Yin et al. 2010; Crhanova et al. 2011; Danzeisen et al. 2011; Sekelja et al. 2012). Certain bacteria may disappear over time or emerge in the intestinal microbiota of older chickens while others remain stable throughout the life (Pourabedin et al. 2015). Firmicutes species are dominant in young chickens while the representatives of Bacteroidetes are most common in adult birds (older than 7 months) (Callaway et al. 2009; Videnska et al. 2014b). In layers, four different profiles of cecal microbiota have been identified from the day of hatching until 60 weeks of age (Videnska et al. 2014b). However, temporal characterization of gut microbiota in poultry varies among studies and needs more frequent sampling and robust sequencing and analyses.

### GUT MICROBIOTA IN POULTRY HEALTH

The gut microbiota is one of the main defense components in the GI tract against enteric pathogens. Disturbance of the gut microbiota-host interaction plays a crucial role in development of intestinal disorders. Significant changes in cecal microbiota have been evident in chickens infected with Clostridium perfringens (Feng et al. 2010; Stanley et al. 2012; Skraban et al. 2013), Eimeria species (Perez et al. 2011; Stanley et al. 2014b; Wu et al. 2014) and Salmonella Enteritidis (Nordentoft et al. 2011; Juricova et al. 2013; Videnska et al. 2013). The chicken gut microbiota has also modulated intestinal gene expression (Yin et al. 2010), T-cell-mediated immunity (Mwangi et al. 2010) and accelerated gut immune system maturation (Crhanova et al. 2011). Furthermore, microorganisms in the gut interact with each other as well as with the host, influencing many physiological functions within the host. Several bacterial phenotypes, more specifically within genera Lactobacillus, Ruminococcus and Clostridium, were associated with performance enhancement (Torok et al. 2008; Stanley et al. 2013a). In the chicken cecum, Clostridium species, particularly certain species in clusters IV and XIVA (these two clusters are predominant in the chicken cecal microbiota), are significant butyrate producers that contribute to growth (Eeckhaut et al. 2011). Butyrate is not only an important energy source for cecal epithelial cells but also inhibits inflammatory responses by acting on proinflammatory cytokines (Eeckhaut et al. 2011). In a metagenomic analysis of cecal microbiota, over 200 non-starch polysaccharide-degrading enzymes and several pathways associated with production of short chain fatty acids (SCFAs) were detected (Sergeant et al. 2014). These SCFAs not only provide energy for the chickens but also indirectly benefit them by lowering cecal pH which prevents growth of pathogens and enhance mineral absorption.

### EFFECTS OF PREBIOTICS ON GUT MICROBIOTA

Manipulation of the gut microbial composition using prebiotic supplementation has been the subject of many investigations. In a comprehensive review by Gaggia et al. (2010), several bacterial culture-based studies have reported higher abundance of lactobacilli and bifidobacteria in the gut microbiota of chickens fed prebiotic supplemented diets. Furthermore, various potential mechanisms have been proposed for health benefits of prebiotic-mediated changes in the gut microbiota (Fig. 2), including competitive exclusion of pathogens (Callaway et al. 2008), production of antimicrobial factors (Chen et al. 2007, Munoz et al. 2012), stimulation of host adaptive immune system (Babu et al. 2012; Yitbarek et al. 2012) and improving gut morphological structure (Chee et al. 2010; Pourabedin et al. 2014). Traditional culture-based techniques have been fundamental to our current understanding of gut microbiota. However, the limited number of cultivable bacterial species has significantly hampered our ability to unravel the full complexity of the gut microbiota. The introduction of macular techniques such as next-generation sequencing technologies has enabled us to study complex microbial populations. Therefore, in this review, we will focus on recent literatures that have used molecular-based techniques to specifically study prebiotic effects on the gut microbiota and the immune system in poultry.

### FRUCTOOLIGOSACCHARIDES AND INULIN-TYPE FRUCTANS

Fructooligosaccharides (FOS) and its longer chain version, inulin, are among the most studied prebiotics in humans and animals. FOS are natural linear polymers, up to 10 monomeric, of β-(1-2)-linked fructosyl units, terminated by one glucose residue. FOS are not hydrolyzed by mammalian or avian digestive enzymes and thereby reach the colon undigested, allowing fermentation by gut microbiota (Roberfroid et al. 2010). Using the PCR-denaturing gradient gel electrophoresis (DGGE) method, Rehman et al. (2008) showed that inulin supplementation (10 g/kg diet) did not change microbial community structure in the jejunal and cecal digesta in broilers, whereas Geier et al. (2009) demonstrated that ileal microbiota was significantly different in FOS-fed broilers (5 g/kg diet) compared to the control. Kim et al. (2011) used species-specific quantitative PCR and indicated that FOS (2.5 g/kg diet) increased the population of Lactobacillus, whereas it restricted the growth of C. perfringens and E. coli in broilers. In the same study, FOS treatment increased the ileal Lactobacillus diversity as shown by DGGE (Kim et al. 2011). In an in vitro study, Babu et al. (2012) investigated the influence of FOS inulin on the ability of the chicken macrophage-like HD11 cell line to phagocytose and kill S. Enteritidis. They found that prebiotic-treated cells had significantly fewer viable intracellular S. Enteritidis than the untreated cells, and this effect was linked to reduced IL-1β-associated macrophage cell death. In contrast, there are pieces of evidence suggesting that some pathogenic E. coli strains can metabolize FOS. A gene cluster, called the fos locus, has been identified in the genome of avian extraintestinal E. coli (ExPEC) that encodes proteins involved in FOS metabolism (Schouler et al. 2009; Porcheron et al. 2011). The products of the gene cluster provided a strong growth advantage for the ExPEC strains to colonize the chicken intestine (Porcheron et al. 2012). Growth of undesirable bacteria can suppress the beneficial effects provided by probiotic-mediated utilization of FOS.
MANNANOLIGOSACCHARIDES

Mannanoligosaccharides (MOS) are mannose-based oligomers linked together by β-1,4 glycosidic bonds. They are naturally found in certain plants, beans and the mannoprotein portion of the cell wall of the yeast Saccharomyces cerevisiae. Because birds do not have enzymes to break down the mannan backbone, this oligosaccharide is believed to reach the lower GI tract undigested. Culture-based studies have indicated that various bacterial strains within the genera Bacteroides, Bacillus and Clostridium produce mannanases as endohydrolases with an ability to cleave β-1,4 manno- pyranside in mannan products (Dhawan and Kaur 2007). However, it should be noted that polysaccharide utilization is a multistep process facilitated by synergistic interactions between widespread members of the gut microbiota (Rakoff-Nahoum et al. 2014). In a study by Corrigan et al. (2011), MOS significantly altered bacterial community structure and composition in broiler chickens as revealed by automated ribosomal intergenic spacer technique coupled with 16S rRNA gene clone library analysis. Kim et al. (2011) investigated the impact of 0.025 and 0.05% of MOS on the ileocecal microbiota of broilers using DGGE and qPCR methods. They showed that 0.05% MOS reduced C. perfringens and E. coli, and increased the relative population of Lactobacillus. More recently, bacterial 16S rRNA pyrosequencing was used to study phylogenetic alterations of cecal microbiota in response to MOS supplementation in broilers (Corrigan et al. 2015). MOS supplementation consistently and reproducibly modified the cecal microbial composition, and increased the number of species within the phylum Bacteroidetes, particularly after 35 days (Corrigan et al. 2015).

The dietary MOS may have greater influences in birds subjected to pathogens or environmental stresses. In E. coli-challenged, transport-stressed turkey pouls, yeast extracts supplemented (1 g/kg) diet increased the number and oxidative burst activity of heterophils, and enhanced disease resistance (Huff et al. 2010). In a study with broiler chickens kept under suboptimal environmental conditions, MOS (1 g/kg) increased cecal bacterial diversity, and promoted growth of Lactobacillus and Bifidobacterium species in the cecum (Pourabedin et al. 2014). Mannose-containing carbohydrates may bind with pathogen lectins and prevent its attachment to the epithelial surface. Mannose-bound pathogens therefore pass through the GI tract without colonization. Whole yeast cell wall supplementation (2 g/kg) also decreased a coccidial infection-induced increase in the cecal E. coli and Salmonella colonization (Shanmugasundaram et al. 2013). Dietary supplementation with a whole yeast cell product modulated chicken immune response by increasing the IFN-γ and reducing IL-10 cytokines mRNA expression of the cecal tonsil. In broilers challenged with C. perfringens, MOS (2 g/kg) resulted in an upregulation of ileal toll-like receptor (TLR)2b, TLR4, IL-12 and IFN-γ, whereas it downregulated cecal tonsil TLR2b expression (Yitbarek et al. 2012). In a study on broilers challenged with Salmonella LPS, MOS (2 g/kg) resulted in a mild immune response which terminated systemic inflammation earlier than subtherapeutic virginiamycin (Baurhoo et al. 2012). In layers, supplementation of S. cerevisiae fermentation products (0.75g/kg) reduced incidence and severity of lesions caused by Eimeria maxima (Lensing et al. 2012). The exact reason for these health benefits is unknown and could be due to the...
indirect effects of prebiotic on the immune system and intestinal epithelial integrity. In addition to MOS, innate immune-modulatory activities of mannobiose supplementation have been reported in broilers (Ibuki et al. 2010). Mannobiose could change the expression of genes related to the host defense, increase IgA production and improve S. Enteritidis clearance (Ibuki et al. 2011). In an in vitro model, phagocytic and Salmonella-killing activities of chicken macrophage cell line (MQ-NCSU) increased following treatment with prebiotic β1-4 mannobiose and these were associated with increased production of hydrogen peroxide and nitric oxide as well as upregulation of genes involved in innate immunity (Ibuki et al. 2011). The underlying mechanisms by which MOS modulate immune responses may be through the cell surface mannose receptor that recognizes both host glycopolymers and microbial glycans, or via mannose binding lectins that trigger and propagate an inflammatory response by initiating a cascade of cytokine expression.

**XYLOOLIGOSACCHARIDES**

Xyooligosaccharides (XOS) are chains of β-1,4-linked D-xylpyranoside units, produced by partial hydrolytic degradation of lignocellulosic materials, commonly arabinoxylans, which are found in abundance in the cereal grains (Carvalho et al. 2013). Chickens lack enzymes required to degrade the glycoside link between xylose monomers; therefore, XOS reach the lower intestine tract and cecum, where they are metabolized by xylanolytic microorganisms. In a study by Courtin et al. (2008), qPCR analysis revealed a bifidogenic effect of wheat-branch derived arabino-XOS (AXOS) at a 0.25% dosage in the ceca of the chickens. Eeckhaut et al. (2008) evaluated administration of two different doses (0.2 and 0.4%) and chain length (average DP of 3 and 9) of AXOS for 5 weeks on Salmonella colonization in chickens experimentally infected by S. Enteritidis at 14 days post-hatch. In their study, AXOS significantly reduced cecal colonization and translocation of S. Enteritidis to the spleen at 3 and 7 day post-infection. By using next-generation sequencing, we recently revealed that XOS supplementation (2g/kg) increased the relative abundance of the Lactobacillus genus in the cecum but the overall microbiota diversity remained unchanged (Pourabedin et al. 2015).

**OTHER POTENTIAL OLIGOSACCHARIDES**

In addition to the above-mentioned oligosaccharides, a few studies have suggested galactooligosaccharides (GOS) (Jung et al. 2008) and soybean meal oligosaccharides (SMO) (Tam et al. 2007) as two prebiotic candidates for chickens. GOS is naturally found in human milk and it has been received a notable attention as a supplement for infant formulas. However, only one study reported the effect of GOS on fecal microbiota in broilers using a bacterial culture assay (Jung et al. 2008). In the study, the abundance of Bifidobacterium species was higher in the birds fed GOS (3g/25 kg diet). SMO has also been found to alter volatile fatty acid concentrations after in vitro fermentation by cecal microbiota of broilers (Tam et al. 2007). An in vivo trial, in the same study, indicated that SMO increased the population of lactic acid bacteria. In two other studies, feed supplementation with a specific prebiotic mixture consisting of galactoglucomannan oligosaccharides and arabinoxylan increased cecal Bifidobacterium and improved the innate immune response in broiler chickens challenged with E. acervulina (Faber et al. 2012a) or S. Typhimurium (Faber et al. 2012b). Apparently, more studies are needed to confirm prebiotic properties of GOS and SMO in chickens.

**CONCLUSIONS AND FUTURE DIRECTION**

Up to now, research on the response of the intestinal microbiota to prebiotic supplementation is still limited in terms of extent and depth. Most associations between observed changes of microbiota and prebiotic supplementation remain at levels of phyla and genera and may not be causative. Nevertheless, the studies have suggested that prebiotics are capable of modulating gut microbiota and the immune interactions in favor of chicken health. At this time, most studies have focused on populations of bifidobacteria and lactobacilli as beneficial bacteria for prebiotics. However, it seems rather simplistic to consider only these genera as beneficial or others such as Clostridium as detrimental. In fact, different strains belonging to other numerous genera may have more profound implications for health than the genera commonly used as prebiotics. For instance, C. butyricum (Yang et al. 2012) and Faecalibacterium prausnitzii have been identified to be dominant in the gut (Torok et al. 2011; Oakley et al. 2013), metabolically active and highly beneficial in models of intestinal disorders (Martin et al. 2014; Zhang et al. 2014). In addition, various species within genera Bifidobacterium and Lactobacillus may induce different functional changes in immune responses, metabolic activities or epithelial barrier integrity (Kleerebezem and Vaughan 2009; Wells 2011). Therefore, it is important for future studies to apply high-throughput sequencing techniques and provide a community-wide analysis of the gut microbiota at different levels of the phylogenetic classification following prebiotic supplementation. In addition, it is now becoming clear that dietary fibers including prebiotics that reach the cecum are metabolized by resident bacteria to yield an enormous range of metabolites besides SCFA with significant physiological functions. Thus, other microbial metabolites such as bile acids and polyamines are worthy to be investigated. Furthermore, considering a high variability in gut microbiota of chickens, it is essential in future studies to analyze larger numbers of samples across different populations, from different regions of the GI tract and from different geographical locations. Use of metagenomics approaches together with metabolite profiling would advance our understanding of the gut microbiota-driven pathways and the role played by prebiotics. This will provide new opportunities for improving gut health and preventing disorders associated with gut microbiota. Finally, how to translate research results to field operation for poultry farms remains non-trivial due to existence of many key differences between poultry research facilities and poultry farms.

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**REFERENCES**


Lan Y, Williams B, Verstegen M, et al. Soy oligosaccharides in vitro fermentation characteristics and its effect on caecal...


