Prebiotics and the poultry gastrointestinal tract microbiome

Steven C. Ricke,*†1 Sang In Lee,*†2 Sun Ae Kim,*†3 Si Hong Park‡,*2 and Zhaohao Shi*

*Center of Food Safety, Department of Food Science, University of Arkansas, Fayetteville, AR 72704; and †Cell and Molecular Biology Graduate Program, Department of Food Science, University of Arkansas, Fayetteville, AR 72701

ABSTRACT Feed additives that can modulate the poultry gastrointestinal tract and provide benefit to bird performance and health have recently received more interest for commercial applications. Such feed supplements offer an economic advantage because they may directly benefit poultry producers by either decreasing mortality rates of farm animals, increasing bird growth rates, or improve feed efficiency. They can also limit foodborne pathogen establishment in bird flocks by modifying the gastrointestinal microbial population. Prebiotics are known as non-digestible carbohydrates that selectively stimulate the growth of beneficial bacteria, thus improving the overall health of the host. Once prebiotics are introduced to the host, 2 major modes of action can potentially occur. Initially, the corresponding prebiotic reaches the intestine of the chicken without being digested in the upper part of the gastrointestinal tract but are selectively utilized by certain bacteria considered beneficial to the host. Secondly, other gut activities occur due to the presence of the prebiotic, including generation of short-chain fatty acids and lactic acid as microbial fermentation products, a decreased rate of pathogen colonization, and potential bird health benefits. In the current review, the effect of prebiotics on the gastrointestinal tract microbiome will be discussed as well as future directions for further research.

Key words: poultry gastrointestinal tract, prebiotics, microbiome, oligosaccharides, non-digestible carbohydrates

INTRODUCTION

Antibiotic growth promoters have been associated with animal production since the 1950s as a dietary means to enhance animal performance and promote health (Dibner and Richards, 2005; Jones and Ricke, 2003; Roe and Pillai, 2003). However, the emergence of antibiotic resistance in pathogens identified as public health risks has led to the curtailment of routine antibiotic supplementation for agricultural use and outright banning in some parts of the world (Casewell et al., 2003; Singer and Williams-Nguyen, 2014; Ventola, 2015; Van Boekel et al., 2017). While efforts continue to establish more precise causal linkages between pathogens and animal and poultry antibiotic use remain of interest, the phasing out of antibiotics in animal production has continued (Argudin et al., 2017; Hurd et al., 2004; Michael et al., 2014; Singer and Williams-Nguyen, 2014). As a result of this shift, commercial emphasis on antibiotic alternatives that recoup at least some of the benefits of antibiotic administration are now being vigorously pursued in the animal and poultry industry. The goal of these efforts is to identify alternatives that not only benefit the animal host in some fashion, but also, if not outright prevent the colonization of foodborne pathogens in the gastrointestinal tract (GIT) at least limit their establishment.

A wide range of feed additives have been explored for potential application in poultry including phytobiotics, organic acids, probiotics, prebiotics, bacteriophage, and bacteriocins among others and these have been extensively discussed in a number of reviews (Callaway and Ricke, 2012; Clavijo and Flórez, 2018; Dittoe et al., 2018; Hume, 2011; Joerger, 2003; Nisbet, 2002; O’Bryan et al., 2015; Patterson and Burkholder, 2003; Ricke, 2003, 2018; Rivera et al., 2015; Vidanarachchi et al., 2005). Some of these alternatives involve some form of a biological agent capable of either specifically inhibiting foodborne pathogens and/or function in a more broad-spectrum antimicrobial manner. However, prebiotics perform more indirectly as substrates for members of the poultry GIT microbial population which in turn respond by increasing in numbers and generating metabolites and other mechanisms that could be considered antagonistic to foodborne...
pathogens in poultry (Kim et al., 2019; Micciche et al., 2018; Ricke, 2003, 2018). As the commercial demand grows for feed amendments in poultry diets that improve GIT health and bird performance, interest in prebiotics has accelerated in the past decade (Alloui et al., 2013; Hajati and Rezaei, 2010; Kim et al., 2019; Micciche et al., 2018; Pourabedin and Zhao, 2015; Ricke, 2015, 2018; Teng and Kim, 2018). In the current review, general properties of prebiotics will be discussed, followed by poultry microbiome responses to prebiotics, and finally the application of microbiome characterization approaches to gain a more in-depth understanding of the poultry GIT microbiome interaction with these compounds.

**PREBIOTICS—GENERAL CONCEPTS**

Collectively, compounds classified as prebiotics were initially defined by Gibson and Roberfroid (1995) as non-digestible food ingredients that promote one or more number of beneficial bacteria in the GIT, enhance GIT health and potentially improve host health. These authors identified several qualifying factors as characteristics associated with prebiotic requirements including among others (1) the prebiotic candidate must be neither hydrolyzed or absorbed in the upper part of the GIT; (2) serve as a selective nutrient source that supports growth and/or metabolic activity of members of the GIT microbial community that could be considered beneficial; and (3) induce luminal or other systemic physiological responses that benefit the host in some fashion. Early on, most candidates that met these criteria to be considered prebiotics by this definition were certain non-digestible carbohydrates such as fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS), and related carbohydrate polymers (Patterson and Burkholder, 2003; Ricke, 2015, 2018). Since the initial description, the definition of what constitutes a prebiotic continues to be refined and evolve into a more inclusive group of candidates (Gibson et al., 2004, 2017; Hutkins et al., 2016; Roberfroid, 2007). For example, resistant starch (RS), lactulose, and other sources have also been considered as potential prebiotics or least compounds that exhibit some characteristics that could be considered prebiotic-like (Bird et al., 2010; Ricke, 2015, 2018; Roto et al., 2015; Gibson et al., 2017; Hutkins et al., 2016).

In addition to improved GIT and host health benefits, prebiotics offer a dietary means to select for GIT bacteria that can potentially serve as a barrier for colonization by foodborne pathogens such as Campylobacter and Salmonella (Kim et al., 2019; Micciche et al., 2018; Ricke, 2015). Traditionally, prebiotics were believed to favor certain beneficial GIT bacteria such as Lactobacillus and Bifidobacterium (Gibson et al., 2004; Roberfroid, 2007). However, the introduction of 16S rDNA-based Next-Generation sequencing (NGS) sequencing has revealed that the poultry GIT microbiome response to dietary prebiotics may involve more members of the GIT microbial community than just a select few (Ricke, 2018). Moreover, the poultry GIT is densely colonized with a complex microbial population, particularly in the cecum (Oakley et al., 2014; Stanley et al., 2014; Svihus et al., 2013). This makes identifying specific mechanisms attributable to individual members of that microbial community and their corresponding microbial physiological responses challenging to delineate. Certainly, the potential for enhancing prebiotic selected GIT microbial production of short-chain fatty acids (SCFA) antagonistic to foodborne pathogens has been suggested as a potential beneficial mechanism (Kim et al., 2019; Micciche et al., 2018; Patterson and Burkholder, 2003; Ricke, 2015, 2018). However, given the complexity of the poultry GIT microbial consortia, it is quite likely that other GIT microbial activities also contribute to a GIT environment that would be considered hostile to foodborne pathogens. Likewise, the range of compositionally different compounds that exhibit prebiotic properties would represent a complex array of substrates for a physiologically diverse GIT microbial population to metabolize. Depending on the metabolite profiles, the resulting fermentation products and concentrations could lead to variable responses of the foodborne pathogens exposed to these metabolites in the GIT.

**IMPACT OF PREBIOTICS ON POULTRY GIT PHYSIOLOGY**

The impact of dietary supplemented prebiotics on the GIT of poultry is likely a function of the chemical composition of the respective prebiotic and metabolic capabilities of the GIT microbiota present. Since prebiotics have been characterized as being indigestible by the host, it is presumed they are hydrolyzed and subsequently utilized by the GIT microorganisms present in various compartments of the avian GIT. Undigested dietary material such as dietary fibers generally transit through the upper parts of the GIT and reach the ceca where they are available as substrates for the resident cecal microbial population (Svihus et al., 2013). The ceca contain a large proportion of obligate anaerobic microorganisms, along with more minor constituents such as methanogens (Saengkerdsup and Ricke, 2014; Svihus et al., 2013). Prebiotics that reach the ceca would likely be utilized by this cecal population resulting in a variety of fermentation products. While quantities of cecal SCFA fermentation products may vary depending upon the diet, in general, they consist primarily of acetate followed by lesser amounts of propionate, and butyrate (Rehman et al., 2007a). Foodborne pathogens such as Salmonella can also reside in the ceca and the production of SCFA would presumably be antagonistic to their presence (Dunkley et al., 2009; Dittoe et al., 2018; Micciche et al., 2018; Ricke, 2003; Van Immerseel et al., 2006). The ceca have several potential roles associated with bird function, including electrolyte and
water reabsorption (Svihus et al., 2013), but it is not clear how the presence of prebiotics might influence these activities. Further studies are needed to determine the interaction between the cecal microbial consortia and cecal physiological functions before and after the introduction of prebiotics.

The upper compartments of the avian GIT such as the crop and the small intestine also harbor microbial populations that would be capable of potentially utilizing specific fractions of some prebiotic compounds (Ricke, 2018). Indeed, SCFA production by resident microbial populations also occurs in these compartments (Rehman et al., 2007a) and the mechanisms of pathogen inhibition by their respective GIT microbiota would likely apply in a similar fashion to what occurs in the ceca. Other mechanisms may come into play as well depending on the prebiotic. For example, competition for limiting nutrients and reduction of colonization by directly binding type 1 fimbrae of pathogens have been noted as potential mechanisms (Teng and Kim, 2018). Identifying which nutrients that might be critical to pathogen establishment remains unclear but intestinal microorganisms have been suggested by Apajalahti and Vienola (2016) to compete with the bird’s intestinal tract for dietary amino acids, particularly in the lower part of the small intestine. Apajalahti and Vienola (2016) suggested that lactobacilli which readily utilize amino acids would be part of this competitive GIT microbiota. Since lactobacilli also can ferment prebiotics, this would suggest that the presence of prebiotics could impact host amino acid utilization. This may be true of non-lactobacilli GIT bacteria as well. Ha et al. (1994) demonstrated with pure culture studies that limiting the concentration of specific amino acids favored chicken cecal bacteria over Salmonella Typhimurium.

Other physiological impacts in the poultry intestine can be associated with prebiotics. Specific prebiotics such as mannans can modulate villi development and structure in the small intestine and increase jejunal enzyme specific activities of maltase, leucine aminopeptidase, and alkaline phosphatase (Iji et al., 2001). Rehman et al. (2007b) concluded that inulin only altered the morphology of jejunal villi of broilers, but not sodium-dependent glucose and glutamine transport. This supports the general observation by Flickinger et al. (2003), that prebiotic impact on intestinal absorption may be both animal species and prebiotic specific. Immune function may also be responsive to the presence of prebiotics. It is becoming increasingly evident that nutrition can influence poultry immune function (Klasing, 2007; Kogut, 2009, 2017; Korver, 2012; Swaggerty et al., 2019). Likewise, prebiotics would be expected to have some impact on the bird’s immune system. Some prebiotics such as MOS are directly interactive as antigens and are considered capable of increasing immune signals in birds (Teng and Kim, 2018). The indirect influence of the immune response via modulation of the GIT microbiota would also be a factor. There is evidence to indicate that the immune system, in general, is interactive with the GIT microbiota (Oakley and Kogut, 2016). Reduction of immunogenic foodborne pathogens in the GIT would undoubtedly be one aspect of altering the immune response, but altering the composition and/or quantity of indigenous microbiota would also likely influence this response (Swaggerty et al., 2019; Teng and Kim, 2018).

In the following subsections, prebiotic groups and their respective association with the avian microbiome will be discussed in more detail.

**POULTRY GIT MICROBIOME: NON-DIGESTIBLE OLIGOSACCHARIDES**

Some of the better-characterized prebiotics include the non-digestible oligosaccharides FOS, GOS, and MOS (yeast-based products) along with the less well known non-digestible oligosaccharides have all been examined for potential use in poultry production. The production, performance, and food safety studies for broilers and laying hens have been extensively reviewed elsewhere (Alloui et al., 2013; Hooge, 2004; Hajati and Rezaei, 2010; Kim et al., 2019; Micciche et al., 2018; Pourabedin and Zhao, 2015; Ricke, 2015, 2016, 2018; Ricke et al., 2017a; Santovito et al., 2018; Teng and Kim, 2018) and will not be discussed in detail here. Origins and structures of these prebiotics are relatively well known. Fructo-oligosaccharides (FOS) are formed from fructose that can be found in various plants such as onion, chicory, garlic, asparagus, banana, artichoke as well as other sources (Flickinger et al., 2003; Ricke, 2015). The enzymatic hydrolysis of lactose by β-galactosidase can be used to generate GOS polymers (Torres et al., 2010; Teng and Kim, 2018). The yeast Saccharomyces cerevisiae serves as a source of MOS as well as fermentation products which can exhibit prebiotic-like properties (Roto et al., 2015; Teng and Kim, 2018).

The prebiotic FOS has been extensively studied for application in humans, companion, and food animals (Flickinger et al., 2003; Ricke, 2015; Teng and Kim, 2018). In addition to meeting the qualifications of a defined prebiotic, FOS can be fermented by both Bifidobacterium and lactobacilli, but this is strain-dependent (Kaplan and Hutkins, 2000; Saminathan et al., 2011). Some of this variation can be linked to genomic differences among strains in carbohydrate catabolic capability. For example, Khoroshkin et al. (2016) used transcriptional analyses based on 10 completely sequenced genomes of Bifidobacterium spp. to reconstruct the complex pathways associated with carbohydrate metabolism by the Bifidobacterium genus. Based on their genome analysis they concluded that most carbohydrate catabolic regulons specific for the host or dietary carbohydrates were somewhat local in the genomes with a limited number of genes being regulated vs. the regulons controlling catabolism of the
sugars sucrose, fructose, lactose, glucose, galactose, and maltose being conserved across most of the genomes.

Examining GIT bacteria at the species level is also essential to consider because even when genera and species of bacteria are the same, preferred substrates for fermentation may vary under different circumstances. Consequently, variation in prebiotic utilization may also be a function of substrate preference by an individual strain or species. For example, while lactobacilli can use many prebiotic carbohydrates, Chen et al. (2018) demonstrated with *Lactobacillus plantarum* that carbon utilization is elicited through carbon catabolite repression allowing preferred carbon sources to be used first followed by less preferred carbon sources. Less is known about the catabolic pathways associated with GOS and MOS. When Saminathan et al. (2011) screened 11 poultry lactobacilli isolates for utilization of GOS and MOS, most were able to grow with GOS as a substrate, but MOS was poorly used. Presumably, there would be similar catabolic regulatory networks associated with other prebiotic oligosaccharides by these same microorganisms as has been recently demonstrated with bifidobacterial utilization of arabino-oligosaccharide utilization (Arzamasov et al., 2018).

Given the complex microbial community present in the ceca, it would be unlikely that lactobacilli and *Bifidobacterium* are the only members of the poultry GIT microbial community capable of fermenting oligosaccharides. This is supported by in vitro work with cecal cultures demonstrating fermentation responses and microbial population shifts reflective of the presence of FOS (Donalson et al., 2008). More recently, Sergeant et al. (2014) using metagenomic analyses of chicken ceca, identified a considerable array of genes encoding polysaccharide- and oligosaccharide-degradation enzymes with at least some occurring as part of overall polysaccharide degradation systems. It is conceivable that several of these polysaccharide degrading systems potentially belonging to a range of different GIT microorganisms would be capable of hydrolyzing at least some of the prebiotic oligosaccharides reaching the ceca.

Identifying the members of the GIT populations that are responding to the introduction of prebiotics has become much more comprehensive with the emergence of 16S gene-based microbiome sequencing (Ricke et al., 2017b). Since the advancements made with NGS have occurred, several studies have used this approach to examine the poultry GIT microbiome response to prebiotics. For example, Kumar et al. (2019) characterized broiler GIT microbial population responses to diets containing low calcium and phosphorus diets in combination with FOS. Ileal and cecal samples were collected, DNA extracted, and after sequencing the data were analyzed using the bioinformatic computational package Quantitative Insights into Microbial Ecology (QIIME) for Operational Taxonomic Unit assignment. Comparisons among diets in the ileal microbiome revealed 75 in common bacterial taxa across birds fed the diets with 8 genera unique to the low calcium/phosphorous plus FOS fed birds compared to 28 genera unique to the positive control group (fully supplemented calcium/phosphorous diet) and the negative control birds (low calcium/phosphorous). Individual genera exclusive to each treatment diet included Clostridia, Blautia, Faecalibacterium, and Pseudomonas for the negative control diets versus Escherichia, Lactobacillus, and Prevotella for the FOS fed broilers. For the cecal analyses, 65 bacterial taxa were shared among the 3 treatments with 7 genera, including Paludibacter, Clostridium, Blautia, Coprooccus, Coprobacillus, Ethanologenes, and Oscillospira associated with the FOS birds.

Kumar et al. (2019) speculated that the increased abundance of Clostridial groups in the negative control diet might be related to the ability of the *Clostridium* genus to generate phytase in response to the low dietary level of phosphorous. However, a metagenomic profiling analysis would need to be conducted to determine if there was an actual increase in the genes associated directly with this enzyme in the microbiome. The appearance of detectable *Lactobacillus* in the ileum of FOS fed birds is supportive of their involvement in FOS utilization and also suggests that their role may be more in the small intestine rather than the cecum. This is consistent with the observation by Kareem et al. (2017) that the presence of the prebiotic inulin exhibited no effect on cecal *Lactobacillus* populations in broilers. Likewise, when Park et al. (2017) compared FOS and GOS fed pasture flock broilers, they concluded that *Lactobacillaceae* were generally underrepresented in the ceca ranging from 2 to 8% relative abundance of the cecal population across all dietary treatment including the control diets that did not contain prebiotic. Age of bird also did not appear to be a factor as this range was consistent at 2, 4, and 6-week-old birds. However, younger ages of birds before 2 wk were not examined, and age does appear to influence microbiome development (Oakley and Kogut, 2016). It remains to be determined how much of a specific prebiotic reaches the ceca quantitatively, but if some of the intact prebiotic does enter the cecum, non-*Lactobacillus* genera may be primarily responsible for its utilization as indicated by the abundance of oligosaccharide enzyme degradation systems detected by metagenomic analyses of the ceca (Sergeant et al., 2014).

**POULTRY MICROBIOME: FERMENTABLE FIBERS**

Besides the well characterized non-digestible oligosaccharides prebiotics FOS, GOS, and MOS, a few other sources have also proposed as possessing prebiotic-like properties. These include RS, fermentable fibers, and other sources of oligosaccharides (Bird et al., 2010; Gibson et al., 2017; Hutkins et al., 2016; Ricke, 2018). The defining key to these proposed prebiotic
sources is their ability to select specific beneficial microorganisms within the GIT microbial community (Gibson et al., 2017). One of the potential prebiotic sources that has received increasing interest are specific components of cereal grains and other fermentable fiber sources (Ricke et al., 2013; Ricke, 2018; Zhuang et al., 2017). Historically, cereal grains have not been viewed as prebiotic sources, but more recent work has indicated that some fractions such as the bran component may behave like a prebiotic when included in an animal diet (Bodie et al., 2019; Ricke, 2018). This is not surprising as many of the grain crops contain fiber and non-starch polysaccharides with varying levels of different beta-glucans present in their cell walls (Knudsen, 2014). Cecal in vitro studies have demonstrated that most are fermentable by cecal microbiota in support of reduced risk of cardiovascular disease (Wang et al., 2016). While less has been done to examine isolated bran fractions in poultry for specific benefits to the bird GIT, there are indications that differences among grains would be detectable. For example, Crisol-Martinez et al. (2017) compared sorghum and wheat-based diets and noted that strains of Lactobacillus crispatus and Lachnospiraceae prevailed in the ceca of sorghum fed chickens while Clostridium leptum phylotypes were more abundant in wheat fed chickens. Although these represent whole grain-based diets, these results suggest that grain source may matter and these differences could also exist in the respective bran fractions among the different grain sources. Ricke (2018) has also raised the point that feed processing such as thermal applications or addition of a feed grade enzyme to the diets may influence the prebiotic properties of a particular grain and its corresponding bran component.

Variation in bran may also be a factor among cultivars within a specific grain source. Rice bran has received considerable attention for its ability to limit Salmonella colonization in mice as well as potential health benefits for human and companion animal diets (Bodie et al., 2019; Goodyear et al., 2015; Ryan, 2011). In poultry, in vitro cecal incubation studies have been conducted to determine if cecal microorganisms can utilize rice bran and if inhibition of Salmonella occurs in the presence of rice bran. Initial work indicated that rice bran was capable of inhibiting S. Typhimurium in cecal in vitro culture, but it was highly dependent on the rice cultivar source of the bran (Rubinelli et al., 2017). The presence of rice bran also led to a decrease in the abundance of the Firmicutes phylum. In addition, an adaptation period was required whereby the cecal cultures were incubated in the presence of the rice bran 24 h prior to inoculating with S. Typhimurium. This modulation of fermentation by rice bran was supported by the metabolic profiles that revealed an increase in metabolites associated with fatty acid metabolism in the presence of rice bran incubations vs. the control with no rice bran. In a follow-up study, Kim et al. (2018) using cecal inocula from birds at different ages (2, 4, and 6 wk) confirmed the rice cultivar specificity for Salmonella inhibition and noted that the greatest reduction occurred with cecal cultures from the oldest birds. Therefore, both grain cultivar source and maturity of the cecal microbiota are potential factors that must be taken into account when assessing prebiotic potential.

CONCLUSIONS AND FUTURE DIRECTIONS

Prebiotics represent feed additives that would potentially select GIT bacteria that potentially benefit the host in several ways, including bird health, prevention of pathogen establishment, and improvement of performance. However, the impact of individual prebiotics may be somewhat variable as there are several chemical and source differences among candidate prebiotics. While it is presumed that prebiotics elicit their impact on specific microorganisms within the GIT microbiota, it remains uncertain which microorganisms are directly involved or how many microorganisms participate in prebiotic metabolism. In addition, microbial communities in different GIT compartments may be involved in prebiotic fermentation. The involvement of the microbiota in the various GIT compartments and how far an intact prebiotic traverses the length of the GIT to reach the cecum may need to be considered in tandem.

Decisions for when to introduce prebiotics to the diet of birds may also be a management strategy to be determined. Given that pathogens such as Salmonella can establish in very young birds (Stavric, 1987), it may be logical to introduce prebiotics reasonably early in the life of a bird. There has been some interest in in ovo administration of prebiotics, and this may represent an effective strategy for early development of a more pathogen antagonistic resident GIT microbial population (Roto et al., 2016; Ricke, 2018). This may be contingent on the type of prebiotic being administered, and effective dosages would need to be determined. How this would influence chick development, and the length of exposure time to the prebiotic could be a factor as well. It would be of interest to compare chick development after in ovo exposure to a prebiotic as to the rate of maturation of the GIT microbiome development post-hatch versus their chick counterparts not given a prebiotic.

Finally, inconsistent performance results need to be overcome in future applications if prebiotics are to gain
universal commercial acceptance. There are no doubt several variables such as housing management, breed of bird, basal feed differences, and external environmental conditions, among others that may very well influence the corresponding response of birds to feed additives. In addition, it is realized that the chicken GIT microbiota are quite complex and therefore it is difficult to predict all of the possible microbial compositional and metabolic responses likely to happen in the presence of a particular prebiotic. Finally, despite the presence of a range of oligosaccharide degrading enzymes byecal bacteria, this is just one aspect of the overall the metabolism that occurs in the ceca as other microorganisms which do not possess such enzymes may still act in concert to utilize some of the hydrolysis products resulting from enzymatic degradation of these polymers as is potentially seen with prebiotics in other GIT microbial ecosystems (Hutkins et al., 2016). Fortunately, the rapid development of sequencing technologies and advancements in bioinformatic pipelines such as QIIME has led to much more comprehensive assessment of GIT microbiome sequence data and the corresponding biological interpretation of that data (Boylen et al., 2019; Ricke et al., 2017b). As these advancements continue it should become possible to delineate some of the complex relationships between the wide range of prebiotic sources and the poultry GIT microbial responses to these prebiotics.

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