

# Prebiotics and alternative poultry production

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**ABSTRACT** Alternative poultry production systems continue to expand as markets for organic and naturally produced poultry meat and egg products increase. However, these production systems represent challenges associated with variable environmental conditions and exposure to foodborne pathogens. Consequently, there is a need to introduce feed additives that can support bird health and performance. There are several candidate feed additives with potential applications in alternative poultry production systems. Prebiotic compounds selectively stimulate the growth of beneficial gastrointestinal

microorganisms leading to improved health of the host and limiting the establishment of foodborne pathogens. The shift in the gastrointestinal microbiota and modulation of fermentation can inhibit the establishment of foodborne pathogens such as *Campylobacter* and *Salmonella*. Both current and potential applications of prebiotics in alternative poultry production systems will be discussed in this review. Different sources and types of prebiotics that could be developed for alternative poultry production will also be explored.

**Keywords:** alternative poultry production, prebiotics, food safety, gastrointestinal tract

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## INTRODUCTION

Foods produced organically or in agricultural systems that are considered natural offer market appeal to some consumers for a variety of reasons including health, food safety, welfare, environmental, and sustainability qualities (Cahill et al., 2010; Lay, Jr. et al., 2011; Mancinelli et al., 2018; van Asselt et al., 2018; Vega-Zamora et al., 2020). However, as van de Weerd et al. (2009) have pointed out for organic animal production, some public perceptions such as improved animal health and welfare remain relatively unproven and will require more comparative studies. This phenomenon is likely true for naturally produced animal products and remains a challenge given the free-range characteristics of some of these operations, such as pasture flock poultry (Ricke and Rothrock, Jr, 2020). Other factors have contributed to the economic growth of alternative agriculture products. For example, interest in locally produced food has become more popular. It has provided further incentive for consumers to pursue alternatively

produced agricultural products and those originating from nearby farms (Feldmann and Hamm, 2015). Public demand and governmental policy choices to move away from antibiotics in food animal production have likely contributed to the heightened demand for organic and natural animal products (Dibner and Richards, 2005; O'Bryan et al., 2008; Diaz-Sanchez et al., 2015a).

Poultry production has not avoided the development of organic and natural product markets. Consequently, alternative poultry production practices have emerged to meet the demand for organic and naturally raised poultry meat and eggs. Organic and pasture flock raised broiler farm management systems have been suggested as a means to accommodate alternative poultry meat production (Fanatico et al., 2009). Pasture broiler flocks typically are raised outdoors with some form of shelter provided during their grow-out period. Given the small size of these operations and limited access to processing facilities, mobile processing units have been designed to accommodate the financial and regulatory requirements for processing pasture-raised broilers (O'Bryan et al., 2014; Mancinelli et al., 2018). In addition to small flock and organic egg production, commercial egg production, in general, has undergone several substantial management changes including the introduction of cage-free and aviary housing systems (Anderson et al., 2009; Mench et al., 2011; Vukina et al., 2014; Ricke 2017; Kidd and Anderson, 2019). Changes in egg layer housing represent different environments than conventional cage layer operations and, thus, potential exposure routes to

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foodborne pathogens not previously identified (Holt et al., 2011).

Alternative poultry production systems represent new challenges for achieving optimal performance while still sustaining acceptable food safety standards. Also, the requirement for natural or organic production standards limits the options for feed supplements that meet these standards. Therefore, some of the feed additives used to improve bird performance or reduce foodborne pathogen load may not be acceptable for organic or natural production systems. However, given the variability in management practices, sizes of flocks, and the diverse environmental elements associated with outdoor exposure, there is a clear need for feed additives that could be used in these types of production systems. Over the years, several feed additives have been suggested and, in some cases, implemented for alternative and organic poultry production including probiotics, prebiotics, and botanical compounds among others (O'Bryan et al., 2008; Sirsat et al., 2009; Diaz-Sanchez et al., 2015b; Ricke, 2015). In this review, the potential for prebiotics and prebiotic-like feed additives for alternative poultry production systems and future applications will be discussed.

## PREBIOTICS - HISTORICAL PERSPECTIVES AND CURRENT CONCEPTS

The original definition of prebiotics as a nutritional component was described a quarter of a century ago by Gibson and Roberfroid (1995) as dietary compounds that were not digested by the host when consumed but could support beneficial bacteria such as *Bifidobacteria* and *Lactobacillus*. Initially, fructooligosaccharides (FOS), galactooligosaccharide (GOS), and mannan-oligosaccharide (MOS) were considered definitive prebiotic compounds. They possessed the characteristics consistent with a typical prebiotic, indigestible by the host, and fermentable by *Bifidobacteria* and *Lactobacillus*. Specific metabolic pathways were identified in these microorganisms that were specifically functional for both the utilization and fermentation of these polymers. Short-chain fatty acids (SCFA) produced during the fermentation of the prebiotic compounds were associated with improved gastrointestinal tract (GIT) health and, in turn, enhancement of host health. In addition, the presence of SCFA served as a barrier to foodborne pathogen establishment in the GIT.

In the following years, both the properties associated with prebiotics and the compounds that qualified as prebiotics have evolved. Part of this progress is linked to technological advances in 16S rDNA microbiome sequencing of GIT microbial communities (Hutkins et al., 2016; Gibson et al., 2017; Ricke, 2018). Characterization of GIT microbiota provided a more comprehensive taxonomic identification of the individual members of GIT microbial populations and diversity comparisons among different GIT microbial communities. The application of metagenomics and metabolomics

further enhanced the understanding of GIT microbial populations by identifying the presence and assigning functional properties of particular genes within the microbial population. Characterizing the GIT microbial communities led to a better understanding of dietary components' responses and refining of prebiotic definitions and identification of additional nutritional ingredients that possess at least some prebiotic properties (Hutkins et al., 2016; Gibson et al., 2017; Ricke, 2018). Consequently, it became apparent that the interactions between dietary components and individual members of the GIT microbial consortia were much more complicated than initially believed (Hutkins et al., 2016; Gibson et al., 2017; Ricke, 2018; Shi et al., 2019).

As a result, expansion of compounds potentially considered to possess at least some prebiotic properties included resistant starches, cereal grain components, and some medicinal herbs among others (Bird et al., 2010; Zhuang et al., 2017; Peterson et al., 2018; Delzenne et al., 2020). Likely, identification and range of additional sources of compounds eliciting at least some prebiotic properties will continue to increase. However, establishing more precise definitions will be elusive since both host responses and the impact on the GIT microbiota can be relatively complex and may not always be consistent. Since the various types of prebiotic and their corresponding mechanisms are potentially different, in-depth profiling of host responses and microbial compositional shifts under different dietary conditions and other variables, such as the host's age, is essential. However, the inability to detect GIT microbial composition changes does not necessarily mean that the presence of the prebiotic does not impact the GIT microbial community. At least some GIT microorganisms are capable of multiple fermentation pathways, which can shift depending on substrate availability. There is potential for metabolic cross-feeding within the microbial community where some microorganisms utilize the hydrolysis products originating from degradation of dietary components by other members of the GIT microbial community (Hutkins et al., 2016; Ricke et al., 2020). Changes in fermentation profiles can, in turn, influence host physiology and the ability of the GIT indigenous microbial population to resist foodborne pathogen colonization. Therefore, when screening prebiotics, several characteristics need to be considered, such as overall host responses and GIT microbial composition. From a GIT microbial perspective, not only should taxonomic composition be considered, but quantification of individual members and metabolomic analyses should be employed to elucidate the potentially more subtle responses occurring in the GIT microbial community in the presence of the prebiotic.

## PREBIOTICS AND POULTRY PRODUCTION

Conventional poultry production has undergone considerable changes with the movement away from antibiotics as feed additives and the subsequently increased

interest in alternative feed additives that provide similar benefits. Identification of feed amendments that can improve feed conversion or broiler growth rate and retain meat quality is the focal point of research efforts (Hume 2011). Likewise, dietary compounds that support layer hen health over a long egg-laying cycle and retain the quality attributes of eggs during egg production are of interest for research development. In addition to performance and health attributes, limiting the establishment of foodborne pathogens in live broilers during grow out as well as dissemination when birds are processed into poultry meat continues to be a significant concern in the poultry industry (Berghaus et al. 2013; Clavijo and Flórez, 2018). Feed additives that target the GIT microbiota and prevent initial colonization of foodborne pathogens can potentially contribute to the overall lowering of contamination (Hume, 2011; Pourabedin and Zhao, 2015; Clavijo and Flórez, 2018). In egg production, the ability to restrict *Salmonella* Enteritidis in susceptible laying hens has been linked to decreases in egg contamination by this pathogen (Ricke, 2017).

There are two general strategies for feed additives to prevent foodborne pathogen dissemination in poultry, namely as an antimicrobial agent against GIT established pathogens or a compound that inhibits initial GIT colonization by pathogens. Some agents such as organic acids, aldehydes, botanical derivatives, and biologicals, including bacteriophages and bacteriocins, serve as antimicrobials. They can potentially reduce foodborne pathogen load in the feed and eliminate foodborne pathogens in the GIT of the bird (Joerger, 2003; Ricke, 2003; Sirsat et al., 2009; Dittoe et al., 2018; Ricke et al., 2019b). The other approach for limiting foodborne pathogen establishment in poultry is by introducing feed additives that alter the GIT microbial community in such a manner that they become more antagonistic to initial colonization by foodborne pathogens. The process can be accomplished by administering probiotics or direct-fed microbials that are live microbial cultures that, once introduced to the GIT, become established as part of GIT microbial community and function to serve as a barrier to incoming foodborne pathogens (Patterson and Burkholder, 2003). A different approach involves the addition of prebiotics that, when consumed as part of the diet, serve as substrates for GIT microorganisms already residing in the poultry GIT that are antagonistic to pathogens. Since prebiotics often support the growth of GIT microorganisms similar to probiotic cultures, combinations of probiotics and prebiotics are referred to as synbiotics. Synbiotics are utilized based on the concept that merging a probiotic culture with a prebiotic compound will potentially help support the establishment of the probiotic organisms by providing specific substrates for utilization by these organisms in the GIT (Patterson and Burkholder, 2003).

Several attributes have been associated with prebiotic supplementation in conventional poultry production. One of the more characterized benefits is the development of GIT microorganisms that decrease the

likelihood of early colonization of pathogens in young birds. In general, young birds are considered to be more vulnerable to enteric pathogens due to their lack of GIT microbiota that can compete with incoming pathogens such as *Salmonella* (Milner and Shaffer, 1952; Stavric, 1987). Consequently, strategies to accelerate the development and increase the complexity of the GIT microbiota in young birds have been introduced. Implementation of feed amendments to young birds such as probiotics or prebiotics to either add microorganisms externally to the GIT or increase proliferation of indigenous GIT bacteria has been developed and marketed commercially (Mead, 2000; Nisbet, 2002; Patterson and Burkholder, 2003; Hume, 2011). Conceptually, once the GIT microbial community's complexity is sufficiently enhanced in the young chick, susceptibility to colonization by pathogens is diminished (Stavric, 1987; Nisbet, 2002). More recent research efforts have been made to modulate the GIT microbiota even earlier in the life of the chick, including in ovo applications (Roto et al., 2016; Peebles, 2018; Teng and Kim, 2018; Rubio, 2019).

Limitation of specific foodborne pathogens establishment in poultry by feeding prebiotics has been discussed in detail for both *Campylobacter* and *Salmonella* in recent reviews (Micciche et al., 2018a; Kim et al. 2019). Prebiotics can limit *Salmonella* establishment either by altering the microbial taxonomic composition and/or fermentation activities in GIT that lead to a hostile GIT environment against *Salmonella* establishment or, in the case of MOS, directly interfere with mannose-specific type 1 fimbriae attachment by *Salmonella* (Micciche et al., 2018a). Nutritional strategies have been developed to extend the utilization of prebiotics further. For example, prebiotics such as FOS have been combined with high fiber diets to alter fermentation and cecal microbial composition in adult laying hen ceca, thus preventing *S. Enteritidis* colonization and infection in susceptible birds such as those undergoing molt (Ricke et al., 2013). The inclusion of the fiber source provided additional substrates for cecal microbial fermentation and influenced cecal microbial composition. *Campylobacter* has proven to be more elusive for developing effective prebiotics (Kim et al. 2019). In summarizing research examining the ability of prebiotics to limit *Campylobacter* in poultry, Kim et al. (2019) noted that reductions were detected in some bird trials but not others. Kim et al. (2019) concluded that this variation could be due to several reasons: differences in detection and quantitation methods of poultry GIT *Campylobacter*. Also, the interaction between *Campylobacter* and the poultry GIT indigenous appears to be complicated and will need to be further understood using advanced molecular techniques to not only detect *Campylobacter* but characterize the GIT microbiota (Indikova et al., 2015; Kim et al., 2019; Ricke et al., 2019a).

Other attributes with prebiotic supplementation in poultry have also been identified (Hajati and Rezaei, 2010; Pourabedin and Zhao, 2015; Ricke, 2018; Teng and Kim, 2018). In general, the support of

beneficial bacteria in the chicken GIT elicits preventative mechanisms against foodborne pathogens through microbial metabolism and results in altered immune responses of the host due to the GIT microbial population changes (Pourabedin and Zhao, 2015; Teng and Kim, 2018). At least some prebiotics can also directly serve as immuno-modulatory agents (Teng and Kim, 2018). The combined influence of feeding prebiotics on the GIT microbial population and corresponding host responses would presumably be reflective in detectable differences in poultry performance. In summarizing potential poultry production benefits from feeding prebiotics, Hajati and Rezaei (2010) suggested that along with limiting pathogens, prebiotics could result in better overall bird performance, improved GIT health and enhanced nutrient utilization accompanied by decreases in environmental pollution and production costs. However, poultry production responses across experimental studies can still be inconsistent and depend upon numerous factors, making predictions and recommendations difficult.

Ideally, analyzing data from multiple independent trials should provide a means to draw at least some general conclusions on effective reoccurring responses to prebiotic supplementation in poultry. For example, Hooge (2004) used meta-analysis to summarize results from global pen broiler trials of birds fed MOS over a range of diets and different environmental conditions that had been conducted for over 10 y. Several criteria were used to select the studies to include appropriate controls (negative control versus positive control consisting of antibiotic supplementation), feeding MOS for the duration of the trial, and proper replication, among others. Based on these analyses, Hooge (2004) detected improvements in feed conversion and body weight compared to the corresponding control. Still, mortalities were significantly lowered in MOS fed birds and were considered the predominant beneficial outcome of MOS supplementation to broilers compared to antibiotic fed birds. Similar analytic approaches would help alternative poultry production systems for evaluating prebiotic supplementation, but comparing trials would be more of a challenge given the diversity in management systems, breeds of birds used, and variation in environmental conditions.

## PREBIOTICS AND ALTERNATIVE BROILER PRODUCTION SYSTEMS

Organic or natural husbandry in the form of free-range or pasture flock raised broilers has become a more popular source of poultry meat (Fanatico et al., 2009, 2013; Rothrock Jr. et al., 2019a). The management of birds raised under these conditions faces many economic and environmental challenges. Nutrition of birds raised under organic conditions has specific requirements for dietary sources originating from organically grown cereal crops and associated ingredients with some exceptions for certain supplements such as the essential amino

acid methionine (Fanatico et al., 2009; Chalova et al., 2016; Burley et al., 2016). Also, the substitution of conventional cereal crops as organic feed sources to more nontraditional cereal crops such as buckwheat has been examined as organic feed sources for broilers (Jacob and Carter, 2008). However, precise nutritional responses are challenging to assess in free-range poultry due to variations in feed management practices (Ricke and Rothrock, Jr., 2020). For example, instead of fully formulated diets, free-range operators can choose to offer diets as separated feed ingredients. Birds self-select individual nutritional components to meet shifting nutrient requirements throughout their grower cycle (Fanatico et al., 2013). The phenomenon is partly due to pasture flock birds' ability to graze over large areas of pasture and consume variable amounts of forages that can meet some of their nutrient requirements (Buchanan et al., 2007; Dal Bosco et al., 2010). While fiber is fermentable by the cecal microorganisms in both young chicks and adult birds, the level of contribution that this fermentation makes to nutrition of the bird remains unclear (Buchanan et al., 2007; Ricke et al., 2013; Ricke and Rothrock, Jr., 2020).

The unrestricted contact between broilers also offers the opportunity for transmission of foodborne pathogens such as *Salmonella* and *Campylobacter* from a variety of environmental sources and nonpoultry vectors outside of conventional poultry housing (Jacob et al., 2008; Van Loo et al., 2012; Trimble et al., 2013a, 2013b; Micciche et al., 2018b; Rothrock Jr. et al., 2019a). Other foodborne pathogens, such as *Listeria*, have also been occasionally isolated from pasture flock raised birds and during subsequent processing (Rothrock Jr. et al., 2017, 2019b). In addition, despite organic and pasture flock broiler operations being antibiotic-free environments, pathogens can still be isolated with antibiotic resistance (Melendez et al., 2010; Thibodeau et al., 2011; Rothrock Jr. et al., 2016). Whether higher pathogen prevalence and concentrations in pasture flock birds versus conventionally housed birds occur is unclear as some studies have demonstrated a lower prevalence of pathogens than conventionally raised birds (Rothrock Jr. et al., 2019a). Rothrock Jr. et al. (2019a) have suggested that this variation is probably due to differences in flock management systems that are dependent on environmental temperature, geography, and wildlife management. In summary, regardless of levels of pathogens present, control measures are needed to limit consumer exposure to foodborne pathogens from meat products generated from broilers raised under organic or natural conditions.

Since organic or natural broilers are grown without antibiotics, there is a need for feed additives that can retain the bird's health and performance and limit the presence of foodborne pathogens. Several interventions have been suggested for application in pasture flock and organically raised broilers including probiotics, prebiotics, organic acids, bacteriocins, antimicrobial peptides, essential oils, medicinal plants, and plant extracts (Griggs and Jacob, 2005; O'Bryan et al., 2008;



Sirsat et al., 2009; Jacob and Pescatore, 2012a; Shi et al., 2019). Prebiotics have been promoted as potential feed amendments for organic and pasture flock broilers to serve as a possible intervention dietary ingredient to limit foodborne pathogen establishment and change the GIT immune system response as well as nutrient availability (O'Bryan et al., 2008; Jacob and Pescatore, 2012b; Ricke, 2015). In addition to the traditional oligosaccharides, chitooligosaccharide (COS), arabinoxylan isomalto-oligosaccharides, and transgalactooligosaccharides have been discussed as potential candidates for alternative broiler production management systems (Jacob and Pescatore, 2012b). However, specific prebiotic studies involving pasture flock or organic broilers remain somewhat limited (Jacob and Pescatore, 2012b; Ricke, 2015).

Early work by Pelicia et al. (2004) compared bacterial and yeast probiotic and prebiotic combinations on free-range Naked Neck breeds of slow-growing broiler performance, GIT development, carcass yield, and meat quality. They reported lower mortalities and higher weight gains for birds fed bacterial probiotic/prebiotic combinations, while the combined bacterial-yeast probiotic-prebiotic improved carcass yield compared to control birds. Pelicia et al. (2004) did not observe GIT development changes based on the weight of all GIT compartments and the length of the duodenum, jejunum, and ileum. In a later study, Hanning et al. (2012) compared Naked Neck broilers with Cornish White Rock cross fast-growing broilers raised on pasture and fed diets supplemented with FOS, GOS, or plum fibers. Growth performance measurements and intestinal villi height and crypt depth were determined every 2 wk over the 8 wk growth period. For fast-growing broilers, FOS increased body weights of birds at 6 wk over the other treatments compared to decreased body weights in GOS fed birds. While addition of plum fibers increased body weights of the 8 wk old Naked Neck birds versus control and FOS fed birds, some variation occurred in villi length and crypt depth related to the feed additive treatment but was dependent on age and breed. For Naked Neck birds, all feed additives supported increased villus length and crypt depth at 8 wk compared to control birds, with GOS being the greatest among all treatments. Such changes in intestinal morphology suggest that the GIT microbial composition and pathogen colonization might be impacted as well when exposed to different prebiotics and may be more profound in certain breeds of birds.

Lilly et al. (2011) compared the performance response and *Salmonella* incidence of organic broilers fed either a *Saccharomyces cerevisiae* cell wall MOS prebiotic, or two different probiotic combinations. Chicks were assigned treatments and raised in pens in a negative-pressure house for the first 21 d then moved to outdoor housing for the remainder of the 49 d. Performance data for d 21 to 49 included body weight gain, feed intake, mortality, and feed conversion ratio corrected for mortality. *Salmonella* occurrence was monitored from environmental samples taken from the feed, litter, and water on d 29 and 50. Performance impact for birds fed MOS

was limited to having the highest body weight gain at d 21, along with birds on one of the probiotic treatments compared to the control birds. Still, by d 49, there were no differences in any of the treatments. Based on collected *Salmonella* samples, the same probiotic combination that influenced body weights at d 21 and the MOS prebiotic was the least *Salmonella* contaminated compared to the other probiotic mixture and the control birds. While environmental *Salmonella* was decreased by MOS supplementation, it would be of interest to examine the GIT contents of these birds at slaughter as well as carcass levels of *Salmonella* contamination to see if this reduction prevailed in the harvested birds. Serovar identification would also be of interest. For example, Melendez et al. (2010) found that *S. Kentucky* was the most frequently isolated serovar from pens, feed, water, and insect traps as well as carcasses in pasture-raised broilers followed by *S. Enteritidis*. This differentiation, along with quantitation, may be useful for determining the relative risk associated with pasture-raised and organic broilers.

Pelicia et al. (2004) suggested that the microbial populations of free-range birds fed prebiotics and probiotics should be examined to determine if these feed additives influence GIT microbial balance. The statement is supported by the intestinal architectural differences among prebiotics observed by Hanning et al. (2012). There is a precedent that the GIT microbiome could be different for pasture flock broilers as the microbial composition of domesticated chickens that have been released and become feral over several generations differs from conventionally raised chickens (Thomas et al., 2019). Given the complexity of the cecal microbial population, this GIT compartment would most likely be the site of detectable differences in GIT microbial populations in pasture flock broilers (Shi et al., 2019; Feye et al., 2020; Ricke and Rothrock Jr., 2020; Rychlik, 2020). When Park et al. (2017) sequenced the cecal populations from pasture flock broilers fed FOS, GOS, or plum fibers, they observed that alpha diversity for all treatments increased as the birds became older. However, cecal microbial populations of FOS and plum fiber-fed birds were more diverse than control birds at 6 wk. Park et al. (2017) also noted a selective enrichment in 6-wk old birds by FOS and GOS for *Faecalibacterium prausnitzii* a known butyrate producer and pectin fermenter. Park et al. (2016) also detected a statistical increase in *Faecalibacterium* abundance for one of the yeast products in Naked Neck pasture flock broilers fed diets supplemented with commercial yeast cell wall prebiotics. While only a limited number of microbiome characterization studies have been conducted on prebiotic fed pasture flock broilers, there appear to be some consistent impacts on cecal populations in birds raised under these conditions. A combination of GIT microbiome compositional characterization and metabolomics, along with host response characterization, is the next potential next step to delineating more definitive mechanistic impacts of prebiotics (Park et al., 2013). This knowledge would help separate bird breed and age

host responses from GIT microbial community shifts in taxa and fermentation. Also, examining microbial populations from other GIT compartments such as the small intestine and the crop may provide additional insight to overall prebiotic impact (Ricke, 2018).

## PREBIOTICS AND ALTERNATIVE EGG PRODUCTION SYSTEMS

The concept of free-range, open access, organic, and cage-free layer hen management operations has evolved into being considered a preferred housing system for commercial egg production for a variety of reasons (Anderson et al., 2009; Mench et al., 2011; Van Hoorebeke et al., 2011; Vukina et al., 2014; Ricke 2017; Kidd and Anderson, 2019). However, there are several challenges associated with these alternative types of egg production. Some, such as nutritional economic costs related to free-range operations have historically been considered a factor when raising pullets (Taylor et al. 1960). Potential differences in exposure to bacterial contamination could also be factors (Holt et al. (2011). De Reu et al. (2005) enumerated eggshell aerobic bacteria and Gram-negative bacterial populations to identify critical contamination sites in conventional cage and organic egg production systems. While the critical contamination point for the caged layer produced layers occurred at the egg processing plant, the most vital bacterial contamination point for organic egg production was the initial contamination in the roll-out nest boxes located on the sidewall of the poultry house. The nest box's impact may be even more specific as the nest lining material has also been shown to influence exterior egg quality (Wall and Tauson, 2013). Samiullah and Chousalkar, (2014) detected higher bacterial loads and lower overall egg quality attributes in eggs collected from free-range layers than caged layers. Pesavento et al. (2017) did not detect foodborne pathogens, including *Campylobacter*, *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes* from free-range and organic eggs collected and sampled from supermarkets. However, they recovered high levels of *Enterococci* spp, with more occurring on the free-range eggs than the organic eggs.

While other foodborne pathogens can be an issue for poultry in general, the risk for exposure to foodborne *Salmonella* infection, particularly *S. Enteritidis* in laying hens continues to be a concern both in the U.S. as well as internationally (Ricke and Gast, 2016; Ricke, 2017; Chousalkar et al., 2018). Based on an extensive literature survey, Denagamage et al. (2015) suggested that the risks for *Salmonella* contamination of table eggs originated from multiple factors. For the risk of *S. Enteritidis* infection in laying hens, Denagamage et al. (2015) concluded that flock size, housing system, and farms with hens of different ages were contributing factors. When Van Hoorebeke et al. (2010) compared the presence of *Salmonella* in conventional caged housing with floor-raised, free-range, and organic layer operations in

different European countries, they did not detect a higher *Salmonella* prevalence associated with these alternative housing types versus conventional cages despite an increased opportunity for oral-fecal contamination. However, Holt et al. (2011) have pointed out that comparisons between free-range and conventionally housed layer hens are confounded by the influence of flock size on *Salmonella* infection. Van Hoorebeke et al. (2011) also offered a cautionary note by pointing out that farm and flock size, age of the building, previous *Salmonella* infections on the farm, among other contributing factors should also be included when focusing on the impact of housing on *Salmonella* occurrence in laying hens. Despite these considerations, Van Hoorebeke et al. (2011) concluded that shifting to nonconventional housing for layer hens was unlikely to increase the risk for *Salmonella* infection. Some differences in *Salmonella* occurrence between free-range and conventionally housed laying hens may exist. For example, while *S. Enteritidis* remains a predominant serovar associated with both conventional and nonconventional housed layer hens, other serovars such as *S. Typhimurium* and *S. Mbandaka* along with others have also been identified with shedding free-range layer hens (Wales et al., 2007; Chousalkar et al., 2016, 2018; Gole et al., 2017).

To counter *Salmonella* infection and subsequent egg contamination, preharvest, and postharvest intervention strategies have been adopted. These strategies propose implementing appropriate biosecurity measures, exercising external vector control, vaccination, disinfection and cleaning in the live bird operations, and proper handling of eggs to prevent *Salmonella* growth (Galiş et al., 2013; Trampel et al., 2014; Chousalkar et al., 2018). Preharvest feed additive interventions include prebiotics and probiotics and have been developed for conventionally housed layers (Galiş et al., 2013; Trampel et al., 2014; Ricke and Gast, 2016; Ricke, 2017). Probiotics have been administered to both young layer chicks and supplements for molting diets in conventional layer hens (Ricke et al., 2013; Ricke, 2016). However, very little research has been conducted with organic and cage-free laying hens. What studies have been done are usually on dietary components that may possess prebiotic-like properties. While not free-range conditions, Bozkurt et al. (2012) demonstrated that MOS supplementation increased eggshell weights in caged layer hens placed in an open-ended shed that exposed the birds to heat stress from ambient temperatures and humidity of western Turkey. Dietary fibers have been touted as potential sources of compounds exhibiting prebiotic-like properties. Layer hens grazing on forages in pastures offer the possibility that there may be some contribution of consumed forage material to layer hen GIT microbial activities (Delzenne et al., 2020; Ricke and Rothrock Jr., 2020). Certainly, layer hen cecal microbiota is capable of fermenting forages such as alfalfa (Ricke et al., 2013). Sozcu and Ipek (2020) examined a lignocellulose product supplemented in feed for layer hens housed in enriched layer cages with perches, nesting areas, and scratch pads. When added at one gram per ton of feed, several

egg production parameters were enhanced, including egg production, weight, quality, immunoglobulin levels, and intestinal architecture. The total aerobic bacterial egg-shell loads were reduced in hens fed the one gram per ton level of the lignocellulose product. While the lignocellulose used in this study was considered insoluble, the fact that GIT morphology was altered suggested that there were interactions between the supplement and the GIT microbial community. It would be of interest to characterize these GIT populations as fibers have been shown to shift cecal fermentation patterns and GIT microbial composition in laying hens (Ricke et al., 2013).

Understanding the nonconventionally reared house-layer hen GIT microbiome could be critical for identifying and optimizing effective prebiotics for these types of birds under these environmental conditions. However, there may be some inherent variability, as evidenced by the much more variable cumulative egg production responses observed by Al-Ajeeli et al. (2018) when they compared Hy-Line Brown laying hens raised either in conventional indoor cages or as free-range. Van Goor et al. (2020) compared cecal microbiomes of laying hens at different egg-laying stages (early: 17 to 23 wk, peak: 25 to 39 wk, and late: 64 to 88 wk) housed at either a conventional or cage-free commercial farm. Cecal microbiome diversity was generally lowest during early lay for hens from both housing systems compared to peak and late egg-laying stages. They also noted the high abundance of Verrucomicrobia in the ceca of free-range layers during peak lay and speculated that this could be related to it being a common soil isolate. However, as they pointed out, the fact that these birds originated from different farms with different management regimens and flock densities limited further interpretation. Adhikari et al. (2020) compared a conventional cage directly and enriched cage layers by placing them in an open-sided house within the same layer house. Two different commercial strains of layers were compared within each housing system, and cecal samples for microbiome sequencing were collected from layers euthanized at 53, 58, 67, and 72 wk of age. Both layer strain and the housing system influenced cecal microbial composition, diversity of microbial communities, and functionality. Given some of the detectable diversity changes reported by Van Goor et al. (2020) in the early laying period compared to later laying periods, more initial time points between the two chicken strains and cage systems could have revealed additional detectable differences.

Thus far, most of the microbiome work has focused on singular studies of free-range or enriched cages alone without conventional cage counterparts that would be directly comparable. As more direct comparisons are made between housing systems, it should become clearer what potential signature GIT microbial populations are consistent for free-range and other alternative housing systems and whether these can be modulated with specific types of prebiotics. Ideally, controlling as many confounding factors as possible, such as similar flock densities and using the same breed of layer, would allow

more precise comparisons. However, more field studies also need to be conducted particularly for free-range layers to determine the impact of environmental factors such as seasonal differences and ambient temperature fluctuations that can lead to heat stress of the birds. Finally, the contribution of unaccounted sporadic dietary intake of nutrients originating from free-range layers grazing forages, consuming insects and other unknown potential nutritional sources must be considered not just in the context of meeting the overall nutritional needs of the bird, but possible underlying biological activities such as prebiotic functions (Ricke and Rothrock, Jr., 2020).

## POTENTIAL PREBIOTIC SOURCES FOR ALTERNATIVE POULTRY PRODUCTION

Pasture flock broilers and layers have access to a diverse diet, particularly when grazing. This fact would suggest that they may develop a fairly complex GIT microbiota with multiple substrate utilization capabilities. This metabolic flexibility could be necessary for dietary sources that may be candidates for providing prebiotic-like functionality to the bird. For example, fiber sources such as forages that free-range birds might graze are accessible to the GIT microbial populations. Indeed, the cecal microbial consortia of the adult broiler and laying hen can ferment dietary fibers sources from forages either in vitro studies or characterization of cecal contents from in vivo feeding studies (Ricke et al., 2013). Presumably, similar cecal fermentation activities occur in flocks grazing on pastures (Ricke and Rothrock, Jr., 2020). The interaction of the cecal microbiota with the fiber content and resulting fermentation would produce SCFA antagonistic to some pathogens, but could also provide some benefit to the host (Ricke and Rothrock, Jr., 2020). Rodrigues and Choct (2018) have pointed out that the inclusion of structural components, such as those derived from whole cereal grains, hulls, and other insoluble fiber sources may contribute to the development of the gizzard by enhancing the holding capacity and grinding capability which in turn benefits the bird by improving GIT functionality.

Cereal grains and specific components such as the bran fraction provide potential sources of prebiotic ingredients (Zhuang et al., 2017). Brans derived from rice and wheat have been shown to exhibit modulation of cecal microbiota composition and metabolic activities in chicken cecal contents (Ricke, 2018). The resulting shifts in cecal microbial populations and fermentation and known to reduce foodborne pathogens such as *Salmonella* (Ricke, 2018). However, this antimicrobial activity can be highly specific, as is the case for rice brans where only specific rice cultivars elicited anti-*Salmonella* activity (Rubinelli et al., 2017). Future studies will need to consider not only the cereal grain source of bran but examine multiple cultivars of a specific cereal grain to identify the optimal cultivar sources of potential



prebiotic-like substrates. The GIT microbiome responses may offer a means to predict which cereal bran components are contributing to the corresponding prebiotic activities.

Cereal grain beta-glucans can also impact fermentation in the crop and result in increased lactic acid concentrations and lowered pH levels (Rodrigues and Choct, 2018). Durant et al. (1999) have demonstrated that enhancing fermentation activities of the lactobacilli in the crop may also be necessary for limiting *S. Enteritidis* in laying hens. While most of the poultry research has focused on conventional cereal grains and their corresponding bran components, opportunities for less utilized cereal grains may offer other sources of brans and beta-glucans that possess prebiotic properties that could be fed to free-range poultry. For example, Jacob and Carter (2008) demonstrated that buckwheat, if supplemented at certain levels, was a viable organic cereal grain source for organic broiler production. Jacob and Pescatore (2012) have suggested that barley is an alternative feed source for poultry. They noted that barley contains higher nonstarch polysaccharides, including beta-glucans, lignin, and cellulose, when compared to corn. It would be intriguing to screen barley grain components for potential prebiotic properties, especially since levels of beta-glucans can vary depending on cultivar differences and variations in growing and harvesting conditions (Jacob and Pescatore, 2012).

Applying external fermentation to feed sources to generate fermentate mixtures of various metabolites may also be a potential source of prebiotics for free-range poultry production. Commercial yeast fermentate preparations have been identified as having prebiotic-like properties that modulate the poultry cecal microbial populations, shift fermentation, and inhibit foodborne pathogens (Roto et al., 2015). Bacterial fermentation of feed constituents is also a possibility. Heres et al. (2003a) used *Lactobacillus plantarum* as an inoculant to ferment a commercial broiler feed mixed with water to generate a liquid fermented feed after incubation with the bacterial inoculant. When *S. Enteritidis* inoculated broilers were compared based on the fermented feed or a dry feed, the fermented feed decreased the susceptibility to *S. Enteritidis* infection potentially due to an increase in ingested lactic acid resulting in a decrease in crop pH (Heres et al., 2003a, 2003b). Other feed sources could also be fermented and fed to free-range poultry. For example, Liu et al. (2018) produced a fermented broccoli product by combining ground stems and leaves with wheat bran, corn flour, and probiotics fermented for 7 d in an anaerobically sealed container. Free-range birds were fed either 5 or 10 % of the fermented broccoli, and performance along with cecal and carcass neck skin bacterial levels were measured. Both levels of fermented broccoli reduced mortalities and the 10% supplementation decreased the numbers *Salmonella* and *Clostridium perfringens* in cecal contents compared to control diet-fed birds. *E. coli* and *Campylobacter* ssp. were reduced by both levels of supplemented fermented broccoli in the cecal contents as well as the neck skin.

Liu et al. (2018) noted that the impact of fermented broccoli might be, in part, due to probiotics and the presence of organic acids and less on the broccoli. Mustafa and Baurhoo, (2016) concluded that unfermented broccoli does not behave as a prebiotic based on the lack of impact on cecal lactobacilli populations and *E. coli*. However, a more comprehensive GIT 16S rDNA microbiome assessment would need to be conducted to determine if nonlactobacilli communities respond to unfermented broccoli and if there are GIT microbial differences between this source of broccoli and fermented broccoli. Agricultural food wastes have also been used as a fermentation substrate for a mixed culture of *Neurospora crassa* and *Lactobacillus plantarum* and produced a feed additive that when supplemented at different levels of the diet improved several egg production parameters in HaiLan laying hens (Liu et al., 2016)

More exotic sources of prebiotics may be options for free-range poultry as well. There are other sources of beta-glucans which could serve as prebiotic candidates. Levine et al., (2018) fed dried *Euglena gracilis* seaweed containing over 50% beta-glucans to broilers challenged with *Eimeria* spp. and demonstrated that algae supplementation could improve GIT immunity and reduce coccidiosis related morbidity. Kim et al. (2018) reported that brown seaweed *Laminaria japonica* elicited a prebiotic impact on rats with enhanced immune response and shifts in rat cecal microbiota. Chitosan oligosaccharides (COS) consist of N acetyl glucosamine with 1–4  $\beta$ -linkages and originate from chitin a polymer found in either fungi cell walls, or exoskeletons of arthropods, and insects (Jacob and Pescatore 2012a; Teng and Kim, 2018). Chitosans have been shown to enhance antibody responses in the presence of vaccines, improve intestinal digestibility, increase growth rate, and increase serum proteins (Jacob and Pescatore 2012a; Teng and Kim, 2018). Invertebrates are consumed by pasture flock birds (Sossidou et al., 2015) and would presumably contribute to the nutrition of the bird. Whether ingestion of insects containing chitin would lead to sufficient breakdown to prebiotic type chitosans is unclear. However, when free-range chickens were fed a *Tenebrio molitor* insect meal, detectable increases in the relative abundance of the cecal genera *Sutterella*, *Ruminococcus*, *Oscillospira*, *Clostridium*, and *Coprococcus* versus the control group not fed the insect meal were observed by Biasato et al. (2018). In a more recent study with *Tenebrio molitor* and *Zophobas morio* insect meal fed to broilers held in chicken coops, Józefiak et al. (2020) reported that a shift in the relative abundance of *Bifidobacterium pseudolongum* and a significant increase in *Lactobacillus agilis* occurred in the birds fed 0.2 % *Zophobas morio* compared to the negative control diet. This observation would indicate that the insect meal containing chitins possesses the ability to modulate the cecal microbiota and, in some cases, may support prebiotic utilizing bacteria. Still, metagenomic-based studies would need to be conducted to determine if genes encoding enzymes capable of hydrolyzing chitosan are present and/or become enriched in the cecal microbial community.



## CONCLUSIONS

Free-range and organic poultry production continues to remain a popular choice for consumers. As the poultry industry evolves to meet consumer demand, it will be critical to develop feed additives that protect bird health, limit mortalities, and minimize foodborne pathogens and poultry disease-causing organisms from becoming established. While many feed additives have been examined in research studies, prebiotics offer a potential intervention approach that can be directly introduced into the feed like any other feed ingredient during feed mixing. There may be a wide range of potential sources of prebiotics such as cereal grains and some forages, which offers versatile applications to meet several different needs for raising free-range poultry. However, more research needs to be done to better understand the mechanisms of various prebiotics by evaluating the effects of prebiotics at the molecular level, while focusing on assessment of GIT microbiome analyses, host GIT responses from metabolism, and immune response standpoint.

## DISCLOSURES

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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