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REVIEW

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Impact of Prebiotics on Poultry Production and Food Safety

Steven C. Ricke*

Center of Food Safety, Department of Food Science, University of Arkansas, Fayetteville, AR

With the phasing out of routine use of antibiotics in animal agriculture, interest has grown for the need to develop feed supplements that augment commercial poultry performance and provide food safety benefits. From a food safety perspective, alternative feed supplements can be broadly categorized as either agents which reduce or eliminate already colonized foodborne pathogens or prevent colonization of incoming pathogens. Prebiotics are considered preventative agents since they select for gastrointestinal microbiota which not only benefits the host but can serve as a barrier to pathogen colonization. In poultry, prebiotics can elicit both indirect effects on the bird by shifting the composition and fermentation patterns of the gastrointestinal microbiota or directly by influencing host systems such as immune responses. Generation of short chain fatty acids is believed to be a primary inhibitory mechanism against pathogens when prebiotics are fermented by gastrointestinal bacteria, but other mechanisms such as interference with attachment can occur as well. While most of the impact of the prebiotic is believed to occur in the lower parts of the bird gastrointestinal tract, particularly the ceca, it is possible that some microbial hydrolysis could occur in upper sections such as the crop. Development of next generation sequencing has increased the resolution of identifying gastrointestinal organisms that are involved in metabolism of prebiotics either directly or indirectly. Novel sources of non-digestible oligosaccharides such as cereal grain brans are being explored for potential use in poultry to limit *Salmonella* establishment. This review will cover the current applications and prospects for use of prebiotics in poultry to improve performance and limit pathogens in the gastrointestinal tract.

INTRODUCTION

Historically, the commercial poultry industry experienced tremendous changes in growth of all phases from the hatchery to broiler and layer farm practices along with meat and egg processing technological advances for long distance retail distribution [1-2]. This is consistent with the global trend of increases in dietary meat protein consumption occurring in parallel with shifts to societies to higher incomes and increased urbanization [3]. As consumer demand has grown, the volume of poultry meat and eggs produced has also expanded to match this rise in retail demand. As a result, commercial poultry operations evolved into vertically integrated large corporations which encompassed all aspects of poultry production from breeder flocks to retail marketing [1]. This rapid ex-

*To whom all correspondence should be addressed: Steven C. Ricke, Center for Food Safety, Department of Food Science, University of Arkansas, Fayetteville, AR 72704; Tel: 479-575-4678, Fax: 479-575-6936, Email: sricke@uark.edu.

†Abbreviations: GIT, Gastrointestinal tract; MOS, Mannanoligosaccharides; SCFAs, short chain fatty acids; FOS, Fructooligosaccharides; GOS, Galactooligosaccharides; NDOs, Non-digestible oligosaccharides.

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pansion in commercial poultry production has required and will continue to depend on advances in bird genetics, nutritional management, processing technologies, and food safety [4-12].

As the number of consumers worldwide continues to grow and economic shifts in improved income occur, the demand for access to quality protein will no doubt continue to increase both domestically and internationally. This will place further emphasis on improving the efficiency and economics of poultry and animal and poultry agriculture. To meet these increased demands on animal production will require the development of feed additive technologies that not only will improve gut health and limit disease but favorably impact overall animal performance. There are several groups of compounds and biological agents being examined and in some cases commercially marketed. In this review on prebiotics and poultry production, the focus will be on the need for alternative feed additives such as prebiotics, types and sources of prebiotics along with a discussion of the research on the interaction with the gut microbiota and the bird host.

RATIONALE AND CURRENT ALTERNATIVE FEED ADDITIVES FOR POULTRY PRODUCTION

In recent years there have been several emerging issues which either have and/or could impact overall efficiency of poultry production and the overall strategic directions that the commercial industries will pursue. For example, there is an increasing need for alternative cereal grain feed sources. This is due to limited availability in countries where corn or soybeans are not primary crops and in other cases because of biofuel diversion of crops such as corn in the United States that has impacted the ability to retain optimal poultry nutritional formulation [13-15]. Certainly, the interest in sustainability and addressing the increasing environmental footprint associated with intensive agricultural practices, such as poultry, has received more interest both from a policy standpoint as well as recommendations for increased research efforts employing methods, such as life cycle assessment modeling [16-17]. This coupled with the increased awareness of animal welfare have led to regulatory and policy changes such as cage-free egg layer farms which have dramatically changed farm management strategies for large scale egg production [18]. A further market driven impact on poultry production has been the rise of organic and natural or free-range poultry flock commercial production systems that avoid the use of antibiotics [2,19-20]. Likewise, the concerns associated with the widespread use of antimicrobials in conventional poultry and animal production and potential linkages to antimicrobial resistance in humans has led to the removal of antimicrobials from commercial operations either as a regulatory edict in Europe or via public demand on retail food markets in the United States [21].

These shifts in regulatory and public preferences to remove antimicrobials and use nontraditional feedstocks has resulted in renewed interest in exploring alternative feed additives that help to retain optimal poultry performance, improve bird health, and reduce foodborne pathogen occurrence [22]. However, as noted for organic and natural systems, animal health can be at risk once these compounds are no longer present and the food products from these systems may represent a food safety risk as well [23-25]. Given the association of intestinal imbalances with the presence of pathogens, a wide range of alternative feed additives have been investigated over the years in an attempt to retain gastrointestinal tract (GIT†) health and promote resistance to pathogen colonization [26]. Strategies for decreasing GIT pathogen loads have included agents that remove or eliminate already colonized pathogens such as botanicals and bacteriophage [27-30]. Prevention of GIT pathogen establishment can include vaccines specific for particular pathogens that stimulates a specific immune response in the bird or direct manipulation of the GIT microbiota either via the administration of external probiotic bacteria that colonize the GIT tract or selection of resident GIT bacteria that serve as barriers to pathogen colonization [9,31-33]. Most of these strategies have been described extensively in previously published reviews and therefore will not be discussed here. The following sections will focus on applications of prebiotics in poultry and recent developments in nutritional management options for poultry production.

PREBIOTICS – GENERAL CONCEPTS AND MECHANISMS

Prebiotics traditionally were represented by a limited set of carbohydrates and related compounds with fructooligosaccharides (FOS), galactooligosaccharides (GOS), and mannanoligoasacchardes (MOS) being among the more commonly employed in animal and poultry research. Fundamentally, these compounds are not utilized by the host animal or human consuming them but can serve as substrates by particular bacteria such as bifidobacteria and lactic acid bacteria [34-35]. For example, not only have analyses of individual bacteria identified specific metabolic pathways associated with these prebiotic compounds, but metagenomic analyses of human ileal mucosal and fecal bacterial populations has revealed the presence of unique prebiotic carbohydrate degradation pathways among the human GIT microbiota [36-38]. Based on this it would appear that numerous GIT bacteria are potentially involved in metabolizing prebi-

otics and this adds to the complexity of understanding mechanistically how they can influence the host and/or inhibit pathogen establishment.

As Alloui *et al*. [33] point out the exact mechanism(s) for pathogen inhibition have not been elucidated as some of their antagonistic activity is dependent on them being metabolized by the GIT microbiota while other interactions may be microbiota independent. The same can probably be said for animal host benefits as the host responses no doubt also exhibit elements of GIT microbiota-dependent and -independent interactions. Prebiotic differences in pathogen and host responses may also be due to the chemical nature of the prebiotic with FOS and related prebiotics being considered primarily fermentable and thus less likely to remain intact in the GIT for long periods of time. Conversely, the yeast-derived MOS can directly decrease GIT pathogens by binding with the flagella of microorganisms such as *Escherichia coli* and *Salmonella* ultimately decreasing their GIT colonization via interference with their attachment to GIT epithelial cells [39]. Yeast mannans have also been shown to act as immune adjuvants and directly initiate immune responses by binding to macrophages and dendritic antigen presenting cells that contain the C-type lectins of the mannose receptor [40].

Much of what is known regarding prebiotics eliciting inhibition against colonization by foodborne pathogens is based on studies conducted either with GIT mixed culture microbiota incubated *in vitro* or characterization of pure culture microorganisms known to be associated with fermentation of prebiotics. Probably among the best characterized properties identified with GIT microbial antagonism of foodborne pathogens is the production of short chain fatty acids (SCFA, primarily acetate, propionate, and butyrate) and lactate during fermentation. Over a quarter of a century ago, Russell [41] hypothesized that some bacteria such as the lactic acid bacteria could tolerate lower intracellular pH levels than their pathogen co-inhabitants such as *E. coli* which strive to maintain a more neutral intracellular pH. Van Immerseel *et al*. [42] suggested that the presence of certain SCFA such as butyrate may down regulate *Salmonella* invasion genes while propionate, but not acetate, can inhibit epithelial cell invasion. However, acetate may elicit other impacts on foodborne pathogens. Fukuda *et al*. [43] reported that increased acetate generated by bifidobacteria in mice inhibited translocation of the Shiga toxin produced by *E. coli* O157:H7 from the GIT lumen to the blood stream. Responses by pathogens to SCFA may also vary somewhat depending on the environmental conditions. For example, Kwon and Ricke [44] demonstrated that *Salmonella* Typhimurium when exposed to SCFA under anaerobic conditions exhibited increased acid resistance. It is known that under anaerobic conditions *Salmonella* can produce SCFA [45] which may explain some of their potential capacity to resist SCFA under GIT conditions.

Since multiple GIT microbiota could be involved with utilizing prebiotics, relative stability, and time of exposure of specific prebiotics may dictate which mechanism(s) are involved in pathogen and host responses. For example, the lower part of the chicken GIT particularly the ceca has been the point of research emphasis for determining GIT microbiota and pathogen responses because of the high level of fermentation that occurs there as well as the fact that it is a primary colonization site for pathogens such as foodborne *Salmonella* [46-50]. However, if FOS polymers are utilized by lactic acid bacteria, then at least in the chicken the primary habitat for GIT lactobacillus is the relatively acidic crop that occurs at the beginning of the GIT [51-52]. This may still be important for control of pathogen colonization since *Salmonella* have been isolated from the crop as well [48,53]. Durant *et al*. [54] demonstrated that when the feed was withdrawn from adult laying hens the crop pH increased and lactobacilli populations decreased in these birds, while *S.* Enteritidis colonization and systemic infection increased suggesting that the crop GIT population serves as a critical barrier to pathogen colonization. It would be of interest to apply labeling techniques to track structural integrity of prebiotics during their transit through the GIT to elucidate the relative stability of specific carbohydrate-based polymeric prebiotics to determine at what point in the GIT they are hydrolyzed and in turn which members of the GIT microbiota are potentially responsive to their presence. Combining this with identifying the resident microbiota population present in each of the sections of the avian GIT may help to develop more effective delivery vehicles for specific prebiotics. This may become particularly important as more complex sources of compounds that elicit "prebiotic-like" activities are introduced to poultry dietary management.

REDEFINING PREBIOTICS AND SOURCES OF NON-DIGESTIBLE OLIGOSACCHARIDES

A limited set of carbohydrate compounds have traditionally considered possessing all the characteristics that define the classic prebiotic and its associated properties when consumed by animals and humans. As more has become understood about the interactions between the GIT microbiota and prebiotic substrates, the classification has expanded to include a variety of oligosaccharides of varying carbon chain length all of which share the common characteristic of not being digestible by the host. These collectively are referred to as non-digestible oligosaccharides (NDOs) and include FOS, GOS, inulin, isomaltooligosaccharide, and xylooligosaccharide, among

others [55-56]. Several of these have been examined in poultry and their impact on the poultry GIT microbiota and pathogen inhibition characterized [57]. A multitude of mechanisms and functions associated with the avian GIT microbiota have been attributed to prebiotics including interaction with the immune system, altering GIT morphology, and competitive exclusion of pathogens [58]. There are also unique examples of prebiotic candidates that derive from compounds that cannot be used by a particular host animal even though other hosts can metabolize the compound. For example, the disaccharide lactose could theoretically be considered a prebiotic in poultry, since neither the bird nor the foodborne pathogen *S.* Typhimurium can use it as a carbon source [59-62]. In the early 1990's, this concept was put in practice when a lactose selected competitive exclusion (CE) microbial consortia was generated from poultry cecal inoculated continuous culture incubations and the combination of lactose, and CE culture was successively administered in birds to prevent *Salmonella* colonization [63-64]. Similar synbiotic combinations (probiotic and prebiotic fed simultaneously) may be possible as more is learned about the chicken's digestibility limits and other nutrient candidates could be used to develop unique synbiotics with other functions beneficial to the bird host.

The concept of lactose supporting a selected cecal microbial population that could be inhibitory to pathogens such as *Salmonella* suggests that identification of the GIT microbiota involved in metabolizing the prebiotic may be an important consideration when screening for candidate compounds. Given the advances made in next generation sequencing and subsequent application for 16S RNA ribosomal gene-based microbiome sequencing, the opportunities to identify which GIT organisms are responding to certain dietary feed amendments including prebiotics has advanced remarkably [65]. As microbiome resolution has improved this has also created somewhat of a dilemma in conceiving a precise definition for prebiotics. Consequently, the definition of prebiotics has evolved over the years since the mid-1990's when it was first proposed as a concept [34]. More recently, Hutkins *et al*. [66] have concluded that prebiotics currently lack a consensus definition but should still essentially elicit some host health benefit. As they and others [65-67] have pointed out this is more likely mediated through GIT microbiota responses to the prebiotic and may not necessarily be due to compositional changes in microbiota population in direct response to the prebiotic, but could be more of a consortia effort involving primary polymer degrading consortia members as well as cross-feeding organisms.

Determining practical sources of prebiotics for farm animal species such as poultry is the key issue. Certainly, the search for sources for NDOs that elicit prebiotic properties could be fairly expansive. Choices for further development become a matter of using representative GIT models to assess microbiota responses and after initial screening further testing in the targeted animal host. In the poultry industry, additional criteria such as costs, management friendliness, and readily available sources for large feed volume use have to be included in the overall vetting process. Given the economics and large quantities potentially required for poultry nutrition, less purified and therefore cruder fractions of NDO sources are potentially attractive. Certainly, plants and forages containing various botanical NDO compounds are possibilities, but recovery of these fractions to achieve full nutritional availability and consistency in quality could be issues for routine use in poultry diets other than special cases such as alternative molt diets for egg layer hens [68]. However, certain cereal grains represent a source that is already a major part of poultry diets, generally contain nonstarch polysaccharides, and are fermentable by adult layer hen cecal microbiota when incubated *in vitro* [69-70]. Cereal grains offer not only high volume sources but are already processed during feed milling into fractions that potentially could serve as sources of NDOs [70-71]. Of particular interest are the nonstarch polysaccharide fractions in the brans recovered from these grains which have been characterized as containing antibacterial and antioxidant properties as well as other properties which may benefit the host [71-72]. The following sections detail the work that has been done with the two of the more extensively studied potential cereal grain prebiotic sources, namely, wheat and rice.

WHEAT DIETS AND FRACTIONS

Wheat milling removes bran and germ fractions prior to flour production for human foods [71]. Early efforts focused on the incorporation of wheat middlings (wheat milling byproduct that excludes wheat flour) into poultry diets particularly as alternative feedstuffs for laying hens to retain commercial egg production levels [73-74]. In the early 2000's wheat middlings were used as a dietary ingredient to induce molting in egg-laying hens to halt egg production and allow hens to rejuvenate before a second egg laying cycle [75]. The primary purpose was to develop a feed-based molting regime that avoided the previous practice of complete feed withdrawal to initiate molting which was associated with systemic infection and egg contamination by *S.* Enteritidis [76-77]. Providing wheat middlings proved to be effective in shutting down egg production and limiting *S*. Enteritidis infection in these hens [75-77]. Follow- up research indicated that the wheat bran fraction could also be used to induce molt without increasing environmental levels of natural occurring *Salmonella* [78]. Specific carbohydrates isolated

from wheat bran may be more inhibitory to *Salmonella* establishment in birds. For example, when wheat bran arabinoxylooligosaccharides were introduced as supplements into diets of broilers infected with *S.* Enteritidis, the 0.4 percent level of the 9 degrees of polymerization version of the carbohydrate led to the reduction in *Salmonella* cecal colonization and translocation into the spleen compared to control birds [79].

This variation in responses suggested that the wheat fractions may be interacting with the layer hen GIT to limit *Salmonella* in some circumstances thus suggesting a role for the cecal microbiota in metabolizing the wheat fractions. This is supported by studies with broilers fed a pelleted diet containing ground wheat supplemented with whole wheat grain and infected with *S.* Typhimurium exhibiting lower *Salmonella* levels in their gizzards and ilea concomitant with lower gizzard pH levels and decreased levels of *Clostridium perfringens* compared to birds not fed whole wheat grain [80]. Wheat bran that is further purified into various carbohydrates may indicate which groups of GIT bacteria are most likely to be influenced. For example, when wheat bran fractions were added as carbon sources to *in vitro* human fecal anaerobic batch cultures, certain fractions supported distinct microbial populations that had been identified by fluorescent *in situ* hybridization [81]. However, even though increased gas production occurred for all additives only the soluble bran increased butyrate production [81]. It would be interesting to conduct similar *in vitro* characterizations of wheat bran fractions with chicken cecal contents, although butyrate production has been observed with wheat middlings anaerobically incubated with layer hen cecal inocula [69].

RICE BRAN

One of the major agronomic crops in the United States is rice, with Arkansas being one of the leading producers of long and medium grain varieties versus California that harvests mostly short and medium grain varieties [82]. Rice is considered one of the major sources of calories for people worldwide [83]. While much of the research focus has been directed toward optimizing rice production and utilization as human food, the byproducts of rice milling and particularly rice bran have received increasing interest as a potential source of nutraceuticals and other health-promoting compounds [83-84] . Human health benefits have been extensively discussed in a previous review [83] and will not be described in detail here. However, it does appear the genetic diversity in rice cultivars may be an important factor in the array of bioactive and phytochemical compounds present in a given rice cultivar [83,85]. Therefore, it would appear to be critical to screen a wide range of cultivars when assessing the potential for the presence of beneficial compounds or determining prebiotic properties.

One of the more consistent properties associated with rice bran is its ability to limit *Salmonella* colonization in the GIT tract and inhibit systemic invasion. Initial work in mice indicated that single rice bran variety supplemented diets fed to mice a week before infection with *S.* Typhimurium resulted in reduced fecal shedding of the pathogen, decreased levels of pro-inflammatory cytokines and increased lactobacillus colonization [86]. When mice were fed different rice bran varieties as supplements in a maintenance diet and subsequently infected with *S.* Typhimurium, certain varieties elicited more protection compared to others [87]. This suggests that chemical composition may sufficiently vary among varieties of rice bran to be detectable in animal infection models. This was confirmed with a follow-up metabolomics study where two rice variety bran extracts were compared for their respective abilities to inhibit *Salmonella* invasion and intracellular growth in tissue culture mouse and porcine cell lines [88]. Their results indicated that distinct metabolite differences were prevalent between the two rice bran extracts and this corresponded to differences in the respective bran extract to inhibit *Salmonella* tissue culture invasion and intracellular growth [88]. Metabolomic analyses of *in vitro* incubations of combined *S*. Typhimurium and a probiotic bacteria *Lactobacillus paracasei* also revealed a greater reduction of *Salmonella* growth in the presence of the rice bran extract and a distinct metabolite profile [89].

Less work has been done in poultry, but recent studies have been conducted with anaerobic *in vitro* cecal cultures to screen brans from different rice varieties on their ability to inhibit *Salmonella*. A key feature of this type of *in vitro* approach was the concept of comparing unadapted versus adapted cecal inocula to the respective dietary supplement added to the batch culture [90]. The adapted version is believed to be more representative of a bird receiving the diet over a period of time allowing its cecal microbiota to adapt to the presence of the feed additive. In the case of rice bran the distinction between the two was whether the cecal inocula were allowed 24 hours (adapted) to ferment the rice bran, before addition of the *S.* Typhimurium marker strain spike or if cecal inocula and *S.* Typhimurium were added at the same time (unadapted). When three rice brans were compared, the Calrose cultivar was most inhibitory to *S.* Typhimurium under adapted conditions and this corresponded with 16S ribosome gene microbiome shifts, and an increase in the quantity of metabolites affiliated with fatty acid metabolism [91]. In a follow-up study with different rice bran cultivars, cecal inocula were collected from 2-, 4-, and 6-week old birds to determine if the age of birds and their corresponding cecal microbiota were a factor in

Salmonella inhibition and if this followed changes in the microbiome [92]. This was based on previous research indicating that the age of bird impacted the diversity of the cecal microbiome with older birds possessing more diverse microbiota [93]. In the rice bran study, the bran supported the most diverse cecal microbiome populations compared to incubations without rice bran added and 2-, 4-, and 6-week rice bran cecal incubations inhibited *S.* Typhimurium but the 6-week responses were the most pronounced [92].

CONCLUSIONS AND OUTLOOK

With the shift away from the routine use of antibiotics in poultry production, interest has grown in alternative feed supplements. Prebiotics in various forms offer a means to modify the GIT microbiota to benefit the bird in multiple ways. However, considerable research remains to understand the mechanisms associated with prebiotic and the avian GIT and optimize their beneficial impact. The development of next generation microbiome sequencing has opened the door to identifying the GIT microbial populations responding to prebiotic administration, but the impact may not always be detectable as a change in microbial composition. Consequently, other approaches such as metabolomics and transcriptomics are being used to gain an in-depth understanding of GIT microbial and host functional responses. Other approaches such as the use of *Salmonella* transposon mutagenesis to determine essential genes associated with the microorganism's responses to changes in GIT in the presence of prebiotics may offer a unique means to identify specific functional impact(s) elicited by the GIT microbiota against this pathogen [94]. This may be particularly important if *Salmonella* serovar differences occur in response to prebiotic modification of the GIT microbiota. However, using prebiotics to control foodborne pathogens other than *Salmonella* such as *Campylobacter* may be more difficult, since *Campylobacter* appears to be much more integrated with the GIT microbiota [95-96].

Finally, identification of novel sources of prebiotics and optimization of their application in poultry remains a high priority. While rice and wheat and their corresponding components including bran appear to be nutritionally beneficial, other cereal grains such as rye are known to be detrimental in their native form and may require simultaneous administration of feed grade enzymes to improve bird response [97]. Adding feed grade enzymes has also been shown to improve nutritional performance and GIT morphology for laying hens fed wheat middlings [98]. This suggests that there may be opportunities to use feed additives such as feed grade enzymes to make the prebiotic elements of certain cereal grains more readily available to the GIT microbiota and expand the range of cereal grains as potential prebiotic sources. Furthermore, the timing of prebiotic administration may be critical as well. There is evidence that the GIT microbiota develops relatively early in the young chick's life cycle, perhaps even *in ovo* [99]. Addition of prebiotics *in ovo* has been explored and this may be a promising means to achieve greater consistency in prebiotic efficacy and immune function modulation [100-102]. In conclusion, the future of prebiotic development for the benefit of poultry production appears to offer numerous opportunities for discovering novel sources and optimizing the efficacy of those compounds currently in use.

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