Chapter

Use of Prebiotics as an Alternative to Antibiotic Growth Promoters in the Poultry Industry

Bruno Solis-Cruz, Daniel Hernandez-Patlan, Billy M. Hargis and Guillermo Tellez

Abstract

Nowadays there is a great concern about antimicrobial resistance (AMR), which has been recognized as one of the most serious global public health threats. Multilateral organizations focused on global health accept the use of antibiotics in animal production as one of the main drivers of AMR, so that many strategies to control this problem have been proposed, resulting in the total ban of antibiotics as growth-promoting agents. On the other hand, this ban has led to an increase in the incidence of bacterial infections or even to the use of antibiotics at therapeutic doses, which could cause a worse scenario of bacterial resistance. Poultry is one of the most commonly exploited species worldwide and a sector that continues to grow and industrialize in many parts of the world, so it was to be expected that a large part of the antibiotics used in animal production was destined to this industry. The reduction or complete abolition of antibiotics in poultry production would have a positive effect in the control of AMR, but this would also have negative economic and public health repercussions, caused by foodborne pathogens and the decrease of the productive parameters. For that, many specific alternatives have been evaluated and marketed, prebiotics being one of the most promising alternatives for the poultry industry.

Keywords: prebiotics, antibiotics, antimicrobial resistance, poultry, intestinal microbiota

1. Introduction

Over recent years, the scientific community has expressed great concern about antimicrobial resistance (AMR), which has been recognized as one of the most serious global public health threats in this century [1]. Nowadays, most multilateral organizations focused on global health accept the use of antibiotics in animal intended for food production as one of the main drivers of AMR infections in human health, adopting national action plans that commit to reduce the indiscriminate use of antibiotics by their members [2–4]. These action plans propose many strategies to control this problem, particularly by encouraging reasonable and limited use of antibiotics in food animal production, particularly those that are considered of critical importance for both human and veterinary medicine. The World Health Organization (WHO) has issued a series of guidelines and resolutions in regard to the use of antimicrobial agents in animal production, among those that stand out being the overall reduction

in the use of all classes of antimicrobials, with the conditional recommendation not to use those that have been classified as critically important for human medicine, as well as the complete restriction for growth promotion and prevention of infectious diseases that have not yet been clinically diagnosed [5], for which some government regulatory agencies have taken action on the use of antibiotics for animal production, resulting in their total ban as growth-promoting agents [6, 7].

It could be expected that the total ban on the use of antibiotics as growth promoters will lead to a decrease in the levels of antibiotic resistance [8]. However, we cannot ignore some issues resulting from this ban, such as the increase in the incidence of bacterial infections which would also increase the use of other antibiotics at prophylactic or even therapeutic doses, accelerating the development of AMR in these pathogens and making it a worse scenario [9, 10]. In addition, some farming practices must be implemented to reduce the use of antibiotics in animal production, such as adequate animal vaccination, good hygiene and husbandry practices, higher animal welfare, and improved breeding programs, which implies an increase in production costs, and it is still not enough to completely reduce the risks of infection [11, 12].

Poultry is one of the most commonly exploited species worldwide, and a sector that continues to grow and industrialize in many parts of the world [13], so it was to be expected that a large part of the antibiotics used in animal production was destined to this industry [14, 15]. Antibiotics have been used in poultry production for therapeutic, prophylactic, or growth promotion purposes, especially in broiler chickens, which has resulted in huge profits for poultry producers [16].

Although it is a fact that the reduction or complete abolition in the use of antibiotics for poultry production would have a positive effect in the control of AMR and public health, this would also have negative economic repercussions, since production costs and, consequently, the prices of the final products, as well as the international trade of poultry products, would be affected [17, 18]. Furthermore, the antibiotic-free production of poultry could imply public health problems caused by foodborne pathogens such as e *C. perfringens*, *E. coli*, *S. aureus*, *Campylobacter spp*., or *Salmonella spp*. [19–21] while increasing production costs caused by bacterial infections, along with the detriment in the health of the birds and the decrease of the productive parameters. Hence, the ban on the use of antibiotics for poultry production, as well as other increasingly popular trends, such as the growth of the organic products market, has forced poultry producers to find viable alternatives with similar benefits to antibiotics. For that, many specific alternatives have been evaluated and marketed, such as enzymes, prebiotics, probiotics, organic acids, dietary fiber, highly available nutrients, herbs, spices, essential oils, plant components, and vaccines [15].

Among all the available alternatives, prebiotics have proven to be promising alternatives for the poultry industry because they are able to pass through the digestive tract, which facilitates and supports the symbiotic relationship between the host and gastrointestinal tract (GIT) microbiota and results in health benefits for the birds [22–24]. Thus, this chapter exposes the use of prebiotics as feed additives in poultry, with emphasis on their beneficial effects on the microbiota composition, their ability to control pathogenic infections, positive changes in intestinal morphology, improved productive parameters, and immunomodulatory effects as possible mechanisms of action, which make them potential alternatives to avoid the use of antibiotics as growth promoters in the poultry industry.

2. Types of prebiotics used in the poultry industry

It is difficult to describe in a few words what a prebiotic is; nevertheless, all definitions agree that these compounds, when administered as feed ingredients, are

resistant to enzymatic digestion and cannot be absorbed, and still they confer a health benefit for the host animal by selectively stimulating the growth, metabolism, and composition of beneficial native bacteria in the GIT and eliminating the pathogenic ones [25–27]. In general, prebiotics share these common properties, but there are some others that are also common among them, including resistance to gastric acidity, selective fermentability by a limited number of potentially beneficial microorganisms, alteration of the GIT microbiota toward a healthier composition, and modulation of the host animal defense system [28].

Although only carbohydrate-based compounds, such as nondigestible oligosaccharides and non-starch polysaccharides, were previously considered as prebiotic candidates, nowadays the prebiotic concept has expanded to "a substrate that is selectively utilized by host microorganisms conferring a health benefit," so that other substances might fit to it, including a diversity of oligosaccharides with varying carbon chain lengths and even polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids [29, 30]. However, to confirm its status as a prebiotic, studies for each candidate must be performed in the target animal species for its intended use, demonstrating its beneficial health effects mediated through the microbiota.

In aviculture, a wide range of prebiotic alternatives have been evaluated, trying to improve the GIT health and resistance against pathogen colonization; nevertheless, all of them have been well characterized, indicating their source, purity, chemical composition and structure, suitable dose, and side effects, and have the status of generally recognized as safe (GRAS). The most commonly used prebiotics in poultry diets are nondigestible oligosaccharides (NDO), including fructooligosaccharides (FOS) and inulin type, mannan oligosaccharides (MOS), xylooligosaccharides (XOS), galactooligosaccharides (GOS), and isomaltooligosaccharide (IMO), as well as some structural carbohydrate components of non-starch polysaccharides (NSP), such as β-glucan [25, 31, 32]. These prebiotics are commonly administered to poultry orally at first hours or days after hatching, either spraying them directly in the feed or by their direct addition in drinking water; but recently, the administration of in ovo prebiotics in chicken embryos has been proposed as a better route of delivery, since the doses of prebiotics used in ovo could be at least 10 times lower than after hatching, with the same beneficial effects as the oral administration [23, 33].

As mentioned above, the main purpose of prebiotics is to modify the intestinal microbiota in a favorable manner for the host animal and induce positive effects, not only in the intestinal environment but also systemically, which is reflected in positive improvements of the productive parameters such as egg production, body weight gain, feed conversion ratio, and mortality index [34–36]. Besides improved host health and productivity, prebiotics have also proven their efficacy to reduce colonization of important pathogens both for poultry production and public health, such as *Salmonella*, *Campylobacter*, *C. perfringens*, and *E. coli* [37–41]. This set of beneficial effects, along with the lower risks of undesirable side effects in the host and the fact that they are cheaper and easier to produce in a large scale than probiotics, make prebiotics an excellent option as an alternative to minimize the use of antibiotics in poultry production, thus contributing to reduce the problem of AMR [42, 43].

3. Mechanisms of action of prebiotics in poultry

There are many mechanisms and functions of prebiotics which have been associated with the poultry GIT microbiota, and it seems that there are several bacteria involved in their use; but there is evidence that other microbiota-independent

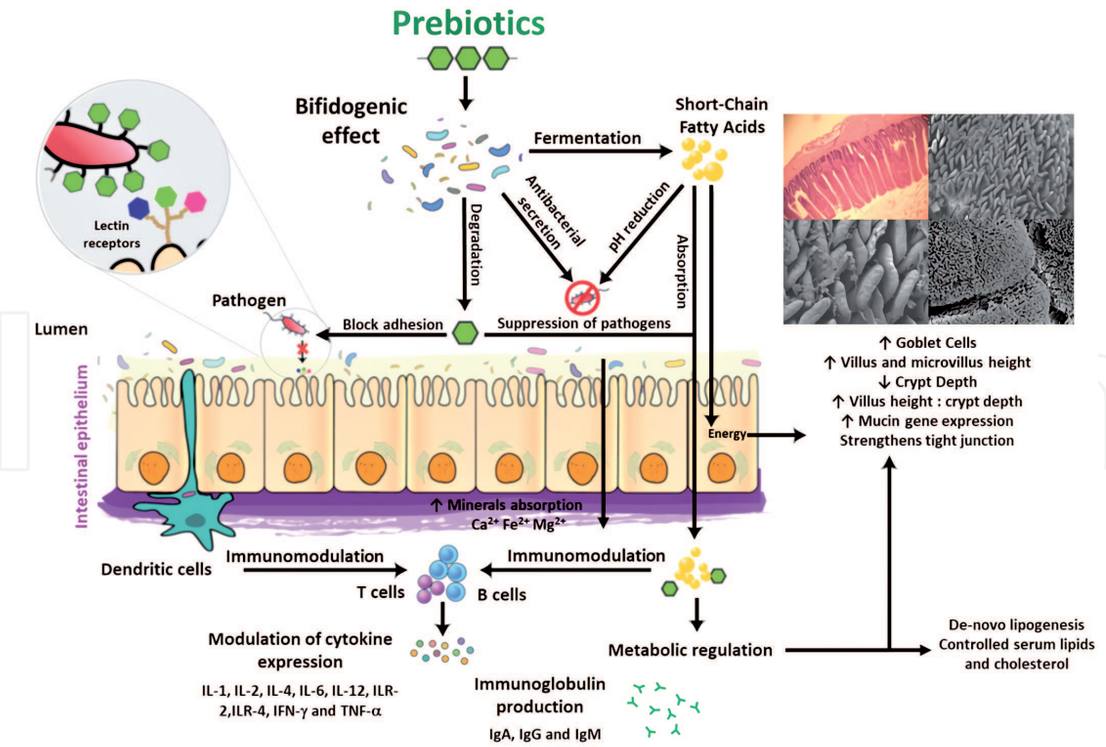

Figure 1.
Some of the potential mechanisms of action of prebiotics in poultry species, showing how they are metabolized by the host microbiota and have positive effects on immunity, gut health, metabolic activity, and pathogen colonization. Modified and adapted from Refs. [25, 45].

interactions also exist [30]. Thus, there is no specific mechanism of how prebiotics influence the host animal health or how they can inhibit pathogen establishment, but all their beneficial effects are the result of a complex set of interactions on the host physiology, including their positive effects on intestinal morphology and microbiota balance, the suppressive effects on the enteric and systemic infections by pathogens due to the immune stimulation, the higher nutrient digestibility, and positive metabolic alterations (**Figure 1**), which result in improved productive parameters, higher quality of food products from poultry origin, general welfare of animals, and ultimately decrease in production cost [42, 44].

3.1 Intestinal microbiota balance

In poultry species, as in almost all the others, the microbiota largely determines the intestinal integrity, functionality, and health, which in turn plays a vital role in nutrient digestion and absorption, immune system development, and pathogen exclusion [46]. Since a symbiotic interaction between host and its microbiota is fundamental to poultry health and production, the alteration of the intestinal microbiota when using prebiotic has been one of the most widely investigated mechanisms of action [25, 32, 47]. Nowadays it is well known that prebiotics alter positively the intestinal microbiota in poultry; however, the exact mechanism (s) and type of interaction involved will depend on the structure of the prebiotic and the host species. Among all the microorganisms that make up the intestinal microbiota in poultry, some particular bacteria, such as *Lactobacillus spp.* and *Bifidobacterium spp.*, have positive effects on intestinal physiology and, thus, are beneficial for the host health. These beneficial bacteria, which have been reported to have higher abundance when chickens are fed diets supplemented with prebiotics, can ferment and metabolize prebiotics, selectively stimulating their proliferation and activity [45, 48, 49], which

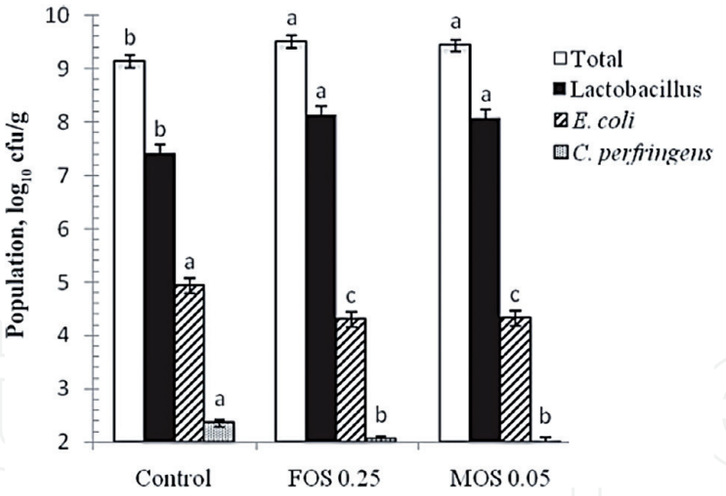

Figure 2.
Intestinal microbiota modifications in the small intestinal content of broiler chickens at 4 weeks of age. Within a bacterial species (or total), bars with different letters (a–c) are different ($P < 0.05$, $n = 8$). Modified from Ref. [40].

can potentially influence the production of short-chain fatty acids (SCFA) and the consequent decrease in intestinal pH, improve the metabolism by increasing digestive enzyme activity and vitamin production and decreasing levels of triglycerides, cholesterol, and odor compounds, and stimulate the immune system that contributes to the inhibitory effects on the growth of pathogenic bacteria [50, 51].

On the other hand, several studies have shown that prebiotics influenced the beneficial intestinal microbiota of broiler chickens while maintaining low levels of potential pathogens in the small intestine and cecal digesta. Addition of FOS as prebiotic to the basal diet (4.0 g/kg) significantly increased the viable count of *Bifidobacterium* and *Lactobacillus* in the small intestinal digesta of male broiler chickens, while the number of *Escherichia coli* was significantly reduced compared to the control group [52].

It has been also reported that feeding 0.25% of FOS and 0.05% of MOS to broilers resulted in an increased diversity and population of *Lactobacillus* and decreased populations of *E. coli* and *C. perfringens* in the ileum, as shown in **Figure 2** [40]. In laying hens, dietary supplementation with different levels of inulin linearly reduced coliform bacteria counts by increasing concentrations of this prebiotic, while 2.0% of inulin achieved significantly increased cecal *Bifidobacterium* counts compared with the control group [53].

A recent study has shown that 3.5 mg of a GOS mixture delivered in ovo had a bifidogenic effect in adult chickens, since the relative abundance of *Bifidobacterium* communities was higher in four sections of intestinal content (duodenum, jejunum, ileum, and cecum), while the values of *Lactobacillus* abundance resulted to be higher in the control group for most of the four sections [32].

3.2 Inhibition of pathogen colonization

The ability of prebiotics in poultry diet to reduce colonization of pathogens results from the combination of several mechanisms occurring in the GIT, from those that are directly related to the selective stimulation of the favorable microbiota to those in which the prebiotics directly affect the pathogens or the host animal in a microbiota-independent manner. To date, it is not possible to define an exact mechanism of prebiotics to reduce pathogenic infections, so more research is required to fully elucidate their exact function and mode of action.

Various potential mechanisms have been proposed by which prebiotics can provide resistance to pathogens, one of the main ones being directly related to the beneficial bacteria in the GIT, such as *Lactobacillus* and *Bifidobacterium*, whose selective growth results in an increased concentration of SCFA, especially acetate, propionate, and butyrate, and lactate during primary fermentation process at the ceca [30]. This is mainly because these bacteria secrete several hydrolases, which monogastric animals cannot, hydrolyzing the carbohydrate-based prebiotics through a fermentation process whose metabolic end products not only contribute to the nutrition of poultry, but they have additional beneficial effects [54]. Since SCFA are the principal luminal anions, and they are relatively weak acids, their increased concentration is correlated with a lower intestinal pH, which in turn is associated with a suppression of pathogens by dissipating the proton motive force across the bacterial cell membrane [55], although it has also been reported that SCFA, butyrate specifically, can downregulate expression of invasion genes in *Salmonella* at low doses [56].

For instance, the effect of 14 or 19 days of 10% dietary lactose administration was evaluated in Leghorn chicks, resulting in a significantly increase of acetic, propionic, butyric, and lactic acid concentration in the cecal contents as compared with the control group; additionally, lactose decreased the pH of cecal contents, with the consequent reduction of the total number of chicks with organ cultures that were positives for this pathogen (**Figure 3**) [57].

Another study was conducted to investigate if changes in SCFA could decrease the numbers of *Enterobacteriaceae* in the ceca of broiler chickens during growth; the authors found a significant negative correlation between the log CFU of *Enterobacteriaceae* and the concentration of acetate, and the undissociated form of acetate, propionate, and butyrate, evidencing that SCFA are one of the mechanisms responsible for the decrease in numbers of these bacteria in the ceca of broiler chickens during growth, while they did not affect beneficial GIT bacteria such as *Lactobacillus* [58].

Nevertheless, there are many other mechanisms by which SCFA may be useful to avoid pathogen colonization in the GIT, such as the increased production of mucin by goblet cells that serves as a physical barrier against pathogens and contributes to their lower colonization [59, 60]. The effect of inulin dietary supplementation at different levels on mucin mRNA expression was evaluated at 21 and 42 days in broiler chickens, and it was found that dietary supplementation of this prebiotic at 10 and 15 g/kg enhanced mucin mRNA expression in the jejunum both days [61]. Moreover, depending on the poultry species, the SCFA provide different levels of the of the total metabolic energy requirements, serving as the preferred energy source of colonocytes and stimulating intestinal integrity [54, 62].

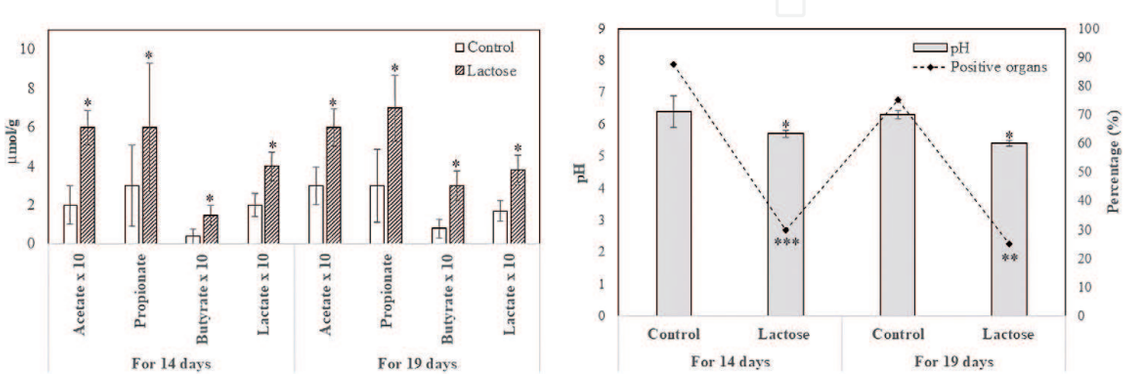

Figure 3.
Effect of dietary lactose (10%) during 14 and 19 days on cecal organic acid concentrations, pH of cecal contents, and Salmonella enteritidis organ invasion in Leghorn chicks. (*) (**) (***) significantly different from controls (P < 0.05) (P < 0.005) (P < 0.001), respectively. Data obtained from Ref. [57].

On the other hand, the natural antipathogen activity of the intestinal microbiota in poultry has been documented by the Nurmi concept of competitive exclusion, also known as "bacterial antagonism" or "bacterial interference," through which beneficial microorganisms compete with potentially pathogenic bacteria for limiting nutrients and attachment sites on the mucosa, or even by the production of bacteriocins like lactocin, helveticin, curvacin, nisin, or bifidocin, which may be destructive to various Gram-positive or Gram-negative intestinal pathogens, particularly *Salmonella*, *Campylobacter*, and *E. coli* [47, 63, 64]. It has been demonstrated that competitive exclusion is potentiated with prebiotics, since they promote growth of beneficial bacteria which are ubiquitous in the host animal and are capable to survive in GIT conditions.

In a study carried out in broiler chicks, the effect of treatments with dietary 7% lactose and 6.3×10^6 of anaerobic organisms, alone or in combination, on cecal colonization by *Salmonella typhimurium* (ST) after 10 and 15 days at different inoculum doses, was evaluated. The authors report that treatment with anaerobes without the addition of lactose did not effectively control cecal colonization of ST, while chickens treated with the combination of anaerobic organisms and lactose were resistant to cecal colonization by this pathogen, concluding that oral administration of only total anaerobes did not function well as competitive exclusion cultures [65].

In another similar study, the inhibitory effect of competitive exclusion and 0.1% concentration of FOS, singly and in combination, on *Salmonella enteritidis* SE colonization of chicks was investigated. Chicks received this pathogen at 7 or 21 days, and then birds from each group were slaughtered at 1, 7, and 14 days after for count of SE in cecal contents. Additionally, quantification of the major cecal microbiota was performed. Results from this study demonstrated the efficacy of CE on chicks 7 days post inoculation with SE, but this efficacy was not clearly demonstrated 21 days post inoculation, indicating that the efficacy of CE to reduce susceptibility to SE colonization is higher on young chicks, while FOS offered protection to chickens particularly in 21-day-old chicks (**Figure 4**). Nevertheless, when FOS was given in combination with a CE treatment, both in the 7- and 21-day-old chicks, a reduction in the number of SE per g of ceca was observed, so that low doses of FOS in the diet of chickens with a CE treatment may result in reduced susceptibility to *Salmonella* colonization. Regarding the intestinal microbiota, few changes in *Bifidobacterium*, *Bacteroides*, and *Lactobacillus* in the cecal contents of treated groups were observed compared with the control group, both 7- and 21-day-old chicks, although when chickens were fed FOS for long times, *Bifidobacterium* and/or *Lactobacillus* of the intestinal flora may increase [39].

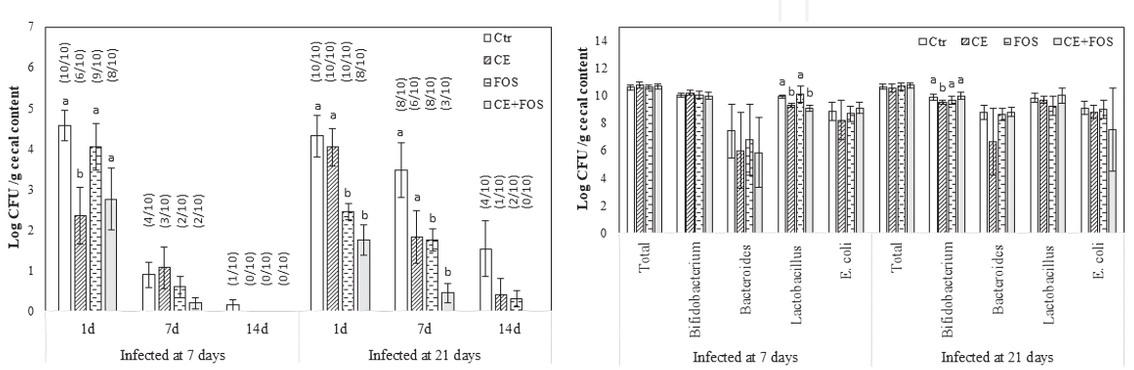

Figure 4.
Effect of competitive exclusion (CE) and FOS (0.1%) on recovery of Salmonella enteritidis from cecal contents of chicks infected at 7 and 21 days. Numbers in parentheses indicate the number of birds positive for SE/birds examined. The right graph shows the effect of these treatments on the major bacterial population of cecal microbiota. Data obtained from Ref. [39].

Some pathogenic bacteria, such as *Salmonella spp., E. coli*, or *Vibrio cholerae*, have mannose-specific lectins (Type 1 fimbriae) on their surface, which recognize glycoprotein receptors rich in mannose on the intestinal cells of the host animal and are key to initiate attachment and colonization [45, 48]. Prebiotics, specifically MOS, can also reduce pathogen colonization by their direct union to the pathogen lectins, avoiding its attachment to the intestinal epithelial cells and, thus, passing through the GIT without colonizing.

This mechanism has been also corroborated in poultry species, both in vitro and in vivo, in two independent studies. In the first study, the in vitro effect of D-mannose, galactose, methyl-α-D-mannoside, and arabinose, on the adherence of *Salmonella typhimurium* to epithelial cells of the small intestine from 1-day-old chicks, was investigated. Authors showed that the small intestine of the chicken has receptors for bacteria with Type 1 fimbriae, and those fimbriae-positive strains of ST adhered significantly better than fimbriae-negative strains. They reported that adherence of ST to chicken small intestinal cells was inhibited more than 90% by methyl-α-D-mannoside and D-mannose and to a lesser extent by arabinose and galactose through the mechanism of blocking [66].

In the other study, the same effect of mannose was demonstrated in vivo. For that, 1-day-old broiler chickens were fed normal drinking water or drinking water supplemented with mannose (2.5% w/v) for 10 days. On day 3, birds were challenged orally with *S. typhimurium* (10^8 CFU), and then the cecal contents were examined on day 10. Results corroborated the blocking action of D-mannose, which could reduce the percentage of chickens colonized by ST from 78 to 28%, 82 to 21%, and 93 to 43%, in three trials [67].

In a more recent study, the ability of MOS from yeast cell walls to decrease the concentrations of enteric pathogens that express Type 1 fimbriae in poultry was evaluated. In the first part of this work, the ability of different enteric pathogens and coliforms to adhere to the MOS was measured in vitro, evaluating qualitatively if agglutination was modified with the presence of fructose, galactose, glucose, and mannose. Results of the agglutination test showed that 5 of 7 strains of *Escherichia coli* and 7 of 10 strains of *Salmonella typhimurium* and *S. enteritidis* agglutinated MOS. Other strains like *S. montevideo*, *S. give*, *S. kedougou*, and *S. dublin* also caused agglutination of MOS, but strains of *S. choleraesuis*, *S. pullorum*, and *Campylobacter* did not lead to agglutination. Nevertheless, agglutination of these Gram-negative bacteria could be inhibited by mannose and fructose, although it took much more fructose to observe the inhibitory responses than mannose. Authors reported that MOS had reduced cecal *S. typhimurium* concentrations by about 25-fold compared to the control group; concentrations of coliforms also tended to be lower when MOS was added to the feed, while concentrations of *lactobacilli*, *enterococci*, and anaerobic bacteria were not affected by treatment; concentration mean values are shown in the upper left graph of **Figure 5**. This tendency to reduce the salmonella concentration was observed during the time after the challenge with the pathogen, as shown in the upper right graph of **Figure 5**. The last part of the study consisted in a similar challenge using *S. dublin*, in which the percentage of prevalence this pathogen was lower in the MOS-treated groups (55.7%) than in the control group (89.8%), while concentrations of the other bacterial populations were not different. Since no changes in cecal parameters were observed with MOS addition, such as a major shift in bacterial populations or changes in pH or SCFA concentrations, which are known to affect salmonella, together with the in vitro agglutination results, authors conclude that adsorption of salmonellae by MOS could be a possible mode of action by which adhesion of these pathogens to the wall is avoided [68].

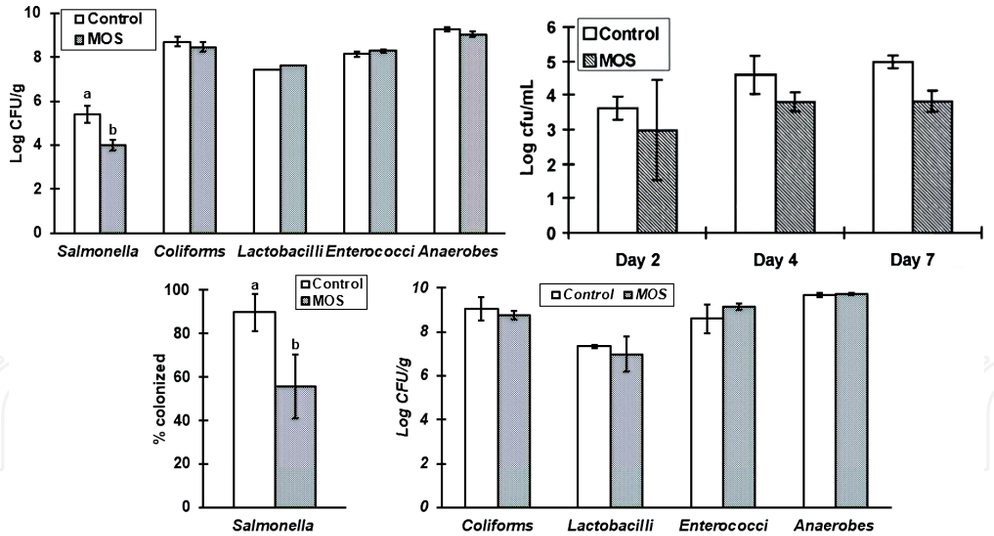

Figure 5.
Upper graphs: Effect of dietary added MOS on concentrations of different bacterial populations (left) and concentration of Salmonella typhimurium at different times after challenge in the ceca of chicks (right). Lower graphs show different bacterial populations (left) in the ceca of chicks challenged with S. dublin and (right) the percentage of birds from which S. dublin was recovered. Within bacterial populations, bars with different letters (a, b) are different (P < 0.05, n = 6). Data were obtained from Ref. [68].

3.3 Intestinal morphology

Another proposed mechanism for health benefits of prebiotics is the improved intestinal morphological structure; several prebiotics have proven their capacity to modify positively intestinal morphology, both on macroscopic (intestinal length) and microscopic (size and density of villi and microvilli and crypt depth) structures of different sections of the intestine in poultry species [52, 69, 70]. Furthermore, an increased number of goblet cells of the intestinal villi have been reported after dietary administration of prebiotics; these specialized cells are responsible for secreting glycoprotein compounds, mainly mucins, which bind pathogenic microorganisms and reduce their adherence to the intestinal mucosa [71]. These morphological changes lead to a higher efficiency of nutrient absorption, since well-developed and functional enterocytes have been associated with increased absorptive area of the intestine [72] but also with an increased activity of the intestinal brush border enzymes and the nutrient transport systems [70, 73].

In turkeys, the dietary addition of two doses of a product based on MOS and β-glucans (1 and 2 lb./ton) on gastrointestinal tract development was evaluated through the measurement of ileal, jejunal, and duodenal morphology of turkey poults at 7 and 21 days of age. Data derived from this study suggest that feed supplemented with MOS and β-glucans could accelerate GIT maturation in turkey poults and was more pronounced in the ileum than in other portions of the small intestine. Ileum villus height, surface area, lamina propria thickness, and crypt depth were enhanced with the prebiotic treatment both on day 7 and 21, in a dose-dependent manner for many of the parameters evaluated, as it can be observed in **Figure 6**. In the jejunum results were consistently higher only for the highest dose of treatment (2 lb./ton) compared with the control group on both days, while in the duodenum results were better for the highest dose on day 7, although intestinal morphology of this intestinal section was not different on day 21. Furthermore, density of neutral, sialomucin, and sulfomucin goblet cells that were taken per intestinal section was also evaluated, showing a very similar tendency than the enteric morphometric

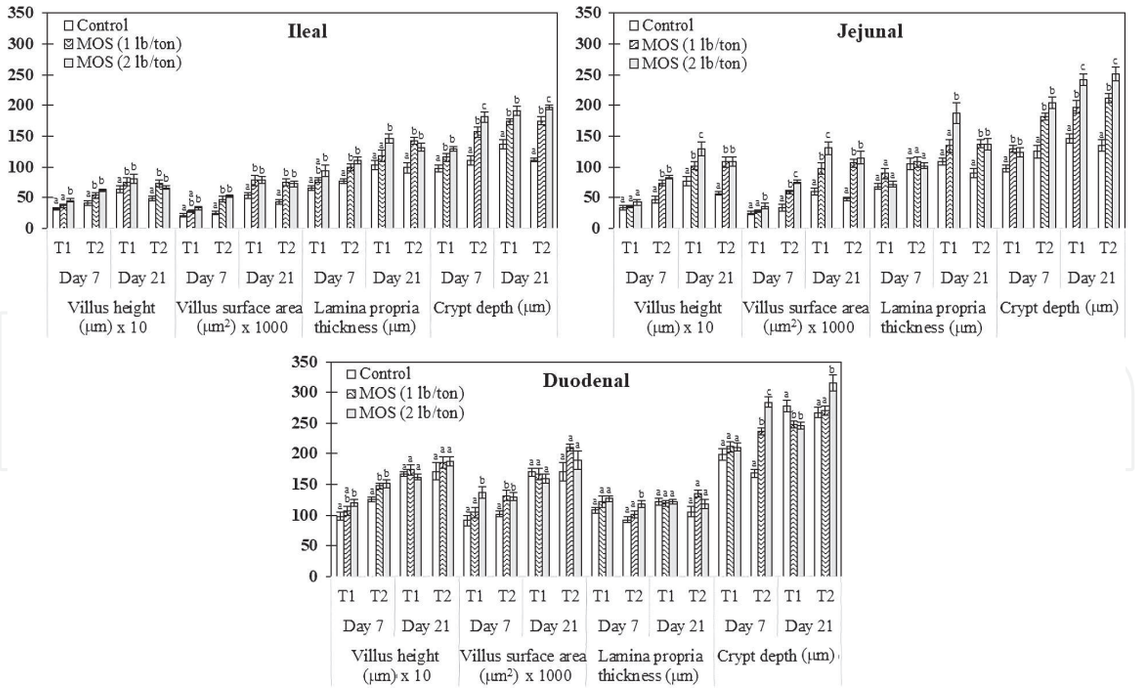

Figure 6.
Effect of MOS dietary addition at two different doses on the ileal, jejunal, and duodenal morphology of Turkey poults at 7 and 21 days of age. Within treatments, bars with different letters (a–c) are different ($P < 0.05$, $N = 9$ birds, $n = 20$ measurements/bird). Data were obtained from Ref. [74].

evaluation, providing evidence of the immunostimulatory effects of this MOS- and β-glucan-based additive, because the numbers of neutral, sialomucin, and sulfomucin goblet cells in the GIT were increased in supplemented poults [74].

Studies have also been conducted whose results demonstrate the beneficial effect of prebiotics on changes at the macroscopic level. A study to evaluate and compare the effectiveness of adding inulin (1%) and oligofructose (1%) to the feed of broiler chickens was conducted, being one of the objectives to evaluate the intestinal length considering the influence of the bird sex. The experiment the experiment lasted 6 weeks, during which the productive parameters were also evaluated. Results from this study suggest that the longer the intestinal length, the better in nutrient absorption which resulted in a heavier body weight, showing correlation coefficients between intestinal length and body weight of 0.68 and 0.74 for the male and female birds, respectively, regardless of the treatments. Oligofructose-treated birds resulted to have a longer intestinal length, especially for the females, although inulin-fed birds also had a longer small intestine than control birds. There were no visible differences in villi density among the males, regardless of the treatments, while for females, the villi from inulin- and oligofructose-treated birds appeared to be denser than those of the controls [69].

3.4 Productive performance

Undoubtedly, one of the main objectives of the use of food additives in the poultry industry is the improvement of productive performance, a major indicator of poultry well-being that is directly tied to efficiency of nutrients utilization and, thus, to the profitability of production. In fact, replacement of antibiotics as growth promoters with prebiotics to observe improvements in poultry performance is the major reason for the researches [28]. As mentioned above, there is no exact mechanism of action for beneficial effects of prebiotics, so that stimulation of poultry performance results from the very complex interactions of all mechanisms previously described, for instance, by decreasing pathogen colonization, since it

has been described that pathogens depress performance by interfering with nutrient digestion, absorption, and utilization; impairment of normal cellular function; negative impact on enzyme activity, epithelial integrity, and function; and diversion of energy for growth to immune response purposes [75]. Prebiotics can potentially stimulate growth performance through increased SCFA production in poultry, mostly acetate, propionate, and butyrate, since they are directly absorbed in the intestine and used as an energy source in tissues and besides that stimulate a higher metabolic activity in the intestine [76, 77]. SCFA may also serve as a potent regulator of insulin homeostasis in the chicken and carbohydrate metabolism, which stimulate the metabolic activity of striated muscle cells, possibly also having an influence on muscle protein synthesis and, thus, growth performance [78]. Beneficial effects of prebiotic on nutrient digestibility of poultry have been also reported, such as improved digestibility of crude protein, fat, dry matter, energy, and minerals [79, 80]. These results have been attributed to an increase in the beneficial microbiota, such as *Lactobacillus*, changes in the intestinal mucosal structure, and improved intestinal health, which result from the morphological changes in the intestine that lead to a higher efficiency of nutrient absorption and a better nutrient transport system, as discussed above [70, 81, 82].

Other prebiotic effects that might influence productive performance of poultry species are alterations on lipid metabolism and mineral absorption [83, 84]. Studies have demonstrated that prebiotic supplements have a positive effect on the mineral metabolism of Ca, P, Zn, Cu, and Fe [85–88], whose intake is influenced by factors such as the lower luminal pH that increases their solubility promotion and thus their passive absorption, changes in the intestinal mucosa and increased absorption surface area, elevated expression of Ca-binding proteins, release of bone-modulating factors, phytate degradation by probiotic bacteria enzymes, and improved overall intestinal health [89, 90]. On the other hand, although no exact mechanisms have been reported for the alteration on lipid metabolism caused by prebiotics, it has been demonstrated that intestinal microbiota play a role in maintaining lipid metabolism [91], so that the increase in bacterial numbers or a change in the composition of the intestinal microbiota might be related to the lipidic alterations. Studies have shown that prebiotics have a positive effect on lipid metabolism in poultry species, such as hypocholesterolemic effect both in serum and eggs, which has been attributed to many reasons. The enhanced production of SCFA results in inhibition of cholesterol biosynthesis in the liver, due to inhibition of the incorporation of colonic acetate into plasma lipids [92]. Another mechanism through which prebiotics may exert hypocholesterolemic effect is via bile acids, since they enter the small intestine and are absorbed and directed to the liver; however, during reabsorption, conjugated bile acids are exposed to intestinal microflora that hydrolyze conjugated bile acids, making cholesterol unavailable for absorption into the circulation [53]. Although it has not been evaluated in poultry, other studies have also suggested that prebiotics may modify gene expression of lipogenic enzymes, with reduced concentration of plasma phospholipids, triacylglycerols, and lipoproteins [93–96]. However, reports of prebiotics on the performance of poultry have been very variable, and often contradictory, as their effectiveness is strongly dependent on the type of prebiotic and the source, dose used, time of consumption, type of diet and interactions with other feed additives, administration route, animal characteristics, hygiene, husbandry conditions, and environmental stress [28, 50].

In a study carried out in White Leghorn hens, the performance parameters were measured to test two prebiotic treatments consumed for 4 weeks, oligofructose (1% w/w) and inulin (1% w/w), during the later part of the first laying cycle. Egg production, cumulative egg weight per bird, and average egg weight for each treatment were calculated weekly. Besides, body weight change, feed consumption, and

feed conversion ratio were also monitored. Results showed that oligofructose and inulin increased weekly egg production by 13.35 and 10.73% and cumulative weekly egg weight per bird by 12.50 and 10.96%, respectively, as compared to the control group. Both prebiotics also improved the feed conversion ratio. Nevertheless, there were no differences in average egg weight, feed consumption, or the percentages of changes in live body weight after 4 weeks, as shown in **Figure** 7 [85].

In another study, the effect of MOS at a dosage of 2 g/kg on growth performance and nutrient digestibility of two cereal-based diets (corn or wheat) in broiler chickens was evaluated, over an experimental period of 21 days. For that, body weight, feed intake, and feed conversion ration were measured at week 1 and weeks 2–3. Also, the ileal digestibility of nutrients was evaluated on day 21. Authors reported that dietary addition of MOS did not affect the body weight gain of birds but increased their feed intake during the first 7 days, while the feed conversion ratio also tended to increase with MOS, regardless of the type of cereal-based diet. Contrary, between 7 and 21 days, dietary MOS improved the growth performance of birds given the wheat-based diet compared to that of birds given the corn-based diet. Regarding the ileal digestibility of starch, the addition of MOS improved it and showed a high interaction with the type of cereal, indicating that this positive effect of MOS was more profound for the wheat diet than for the corn diet.

3.5 Enhancement of immune system

Currently, much research has focused in modulation of the immune system by the use of prebiotics, which results from the interaction of all the mechanisms mentioned above, so that it is not an isolated mechanism. A multitude of mechanisms and functions associated with the immunomodulatory effect of prebiotics have been reported, by the activation of genes and pathways implicated in immune processes [25]. It has been cited for many authors that the use of prebiotics in poultry diets improves bird's immunity through the selective growth of beneficial microbiota, resulting in an increased production of a variety of substances, such as bacteriocins and SCFA, that, in addition to being able to inhibit growth of pathogens, play a role in signaling pathway of immune system [97–99].

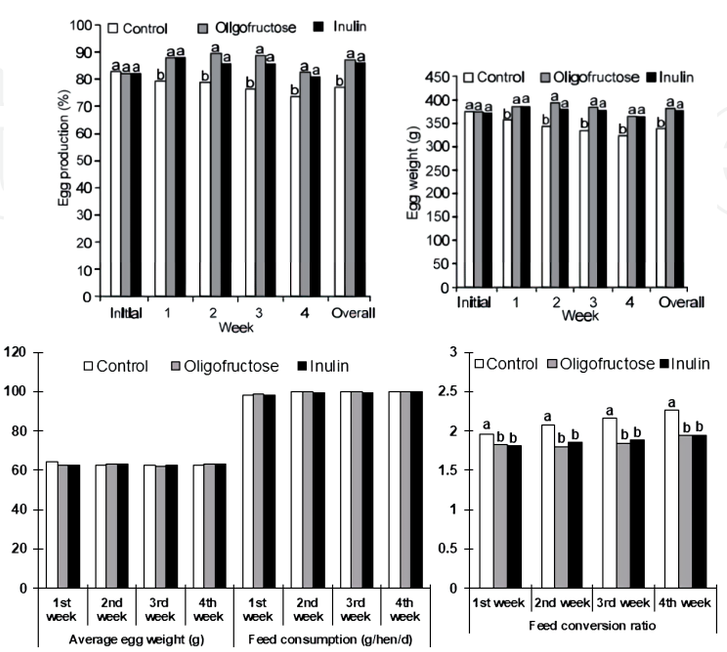

Figure 7.
Performance parameters as affected by dietary oligofructose and inulin in laying hens. Within treatments, bars with different letters (a, b) are different (P < 0.05, n = 10). Data were obtained from Ref. [85].

The chicken gut microbiota, especially *Lactobacillus* and *Bifidobacterium*, has also been reported to modulate intestinal gene expression, T cell-mediated immunity, and accelerated intestinal immune system maturation, by influencing the intestinal epithelium to produce antimicrobial peptides and cytokines such as IL-12, IFN-γ, IL-10, IL-1β, and TNF-α; modulating the immune system through enhancement of phagocytosis and proliferation of immune cells such as macrophages and monocytes; enhancing production of IgA, IgM, and IgG, reactive oxygen species, and reactive nitrogen species; and proliferating natural killer cells, CD3, CD4, and CD8 T cells [25, 47, 75, 100]. Some prebiotics have shown to increase the production of secretory IgA in the intestine, which inhibits the attachment and penetration of bacteria in the lumen, increases the production of mucus, and prevents inflammation that could cause epithelial tissue damage [40, 42].

On the other hand, as previously mentioned, prebiotics can inhibit pathogen colonization, decreasing detrimental molecules produced by pathogenic bacteria, which act as exogenous signals called pathogen-associated molecular patterns (PAMPs). These PAMPs can be recognized by pattern recognition receptors (PRR)

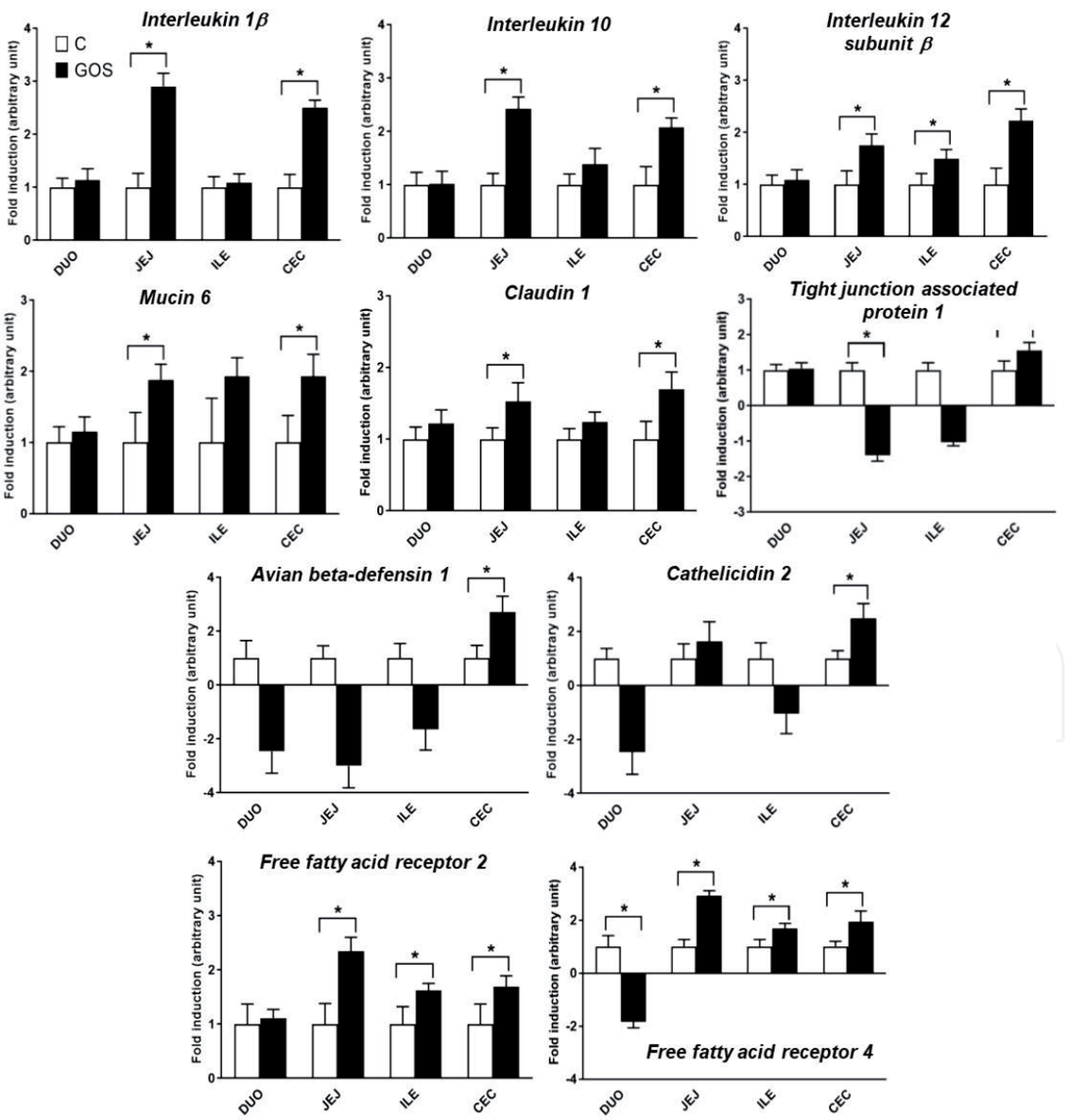

Figure 8.
Relative mRNA expression of immune-related (cytokines and host defense peptides) and barrier function (mucin and free fatty acid receptors) genes in different segments of intestinal mucosa in chickens injected in ovo with GOS. Asterisk indicates pair-wise significant differences ($P < 0.05$, $n = 10$). Graphs were obtained from Ref. [32].

expressed on the surface of epithelial cells, macrophages, mast cells, and dendritic cells, including toll-like receptors and NOD-like receptors, and once recognized are activated, producing cytokines for the regulation of further innate immune responses [45]. Although little data show direct effects of prebiotics on immune function, some studies have indicated that prebiotics have an improved response to salmonella vaccine, which could be because prebiotics can act as nonpathogenic antigens themselves, being recognized by receptors of immune cells, which consequently modulate host immunity beneficially [45, 101].

Immunomodulatory effect of prebiotics has been evaluated in vitro and in vivo. For instance, in an in vitro study, the influence of a FOS-inulin prebiotic at 200 μg/mL on the ability of the chicken macrophage HD11 cell line to phagocytose and kill *Salmonella enteritidis* was tested. The influence on their ability to express selected inflammatory cytokines and chemokines, such as for IL-1β, lipopolysaccharide-induced TNF factor (LITAF), C-C motif chemokine ligand 4 (CCL4) and inducible nitric oxide synthase (iNOS), and nitric oxide production, was also evaluated. Results showed that phagocytosis of SE by macrophages was not affected with the FOS-inulin treatment, but there was a significant reduction of viable intracellular SE in macrophages treated with the prebiotic. On the other hand, prebiotic treatment did not influence the nitric oxide production, thus suggesting that the FOS-inulin-mediated bacterial clearance was not mediated by this compound. Similarly, prebiotic treatment has no influence on expression of LITAF, CCL4, nor iNOS; however, IL-1β expression was significantly lower in macrophages treated with FOS-inulin, suggesting that this prebiotic can modulate the innate immune system by preventing IL-1β-associated macrophage cell death [102].

In a more recent study, GOS prebiotic was in ovo administered to evaluate the modulation of chicken intestinal microflora and demonstrate the molecular responses of the host animal. The study was performed on meat-type chickens, with 3.5 mg GOS delivered by in ovo injection on day 12 of egg incubation, and the analysis of microbial communities and mucosal gene expression was performed at day 42 post-hatching. Results showed that GOS increased the relative abundance of *Bifidobacterium* in the cecum. GOS also upregulated cytokine and barrier function genes in the jejunum and cecum, host defense peptides in the cecum, and free fatty acid receptors in the jejunum, ileum, and cecum, as shown in **Figure 8**, so that it has been demonstrated that GOS prebiotics have a bifidogenic effect in adult chickens, modulating gene expression related to intestinal immune responses and gut barrier function [32].

4. Conclusion

Due to the great concern about AMR, it is imperative to avoid the use of antibiotics as growth promoters and look for effective alternatives that can help poultry production to improve the welfare of the poultry birds, performance, and production costs. As a result of all the studies that have been carried out, we can conclude that dietary addition of prebiotics has a positive effect on poultry production, highlighting the improvement of intestinal health, immune system, control of pathogens, and performance parameters, which are achieved through a series of interrelated mechanisms and interactions involving interactions between the organisms of the intestinal microbiota and the microbiota with the host animal. Nevertheless, effectiveness of prebiotics will depend on many factors, like the type of supplement, doses, composition of the basal diet, animal characteristics, and environmental condition, showing variable effects on poultry species, so that it is necessary to determine conditions under which prebiotics are effective

and elucidate the mechanisms(s) of action involved, ensuring their effective use. Many studies have elucidated mechanisms involved in the effectiveness of prebiotics, but we believe that there is still information that remains to be discovered or that must be confirmed, including the identification of new prebiotics and their application in the poultry industry, for which we can take hold of the emerging analysis technologies.

Acknowledgements

This research was supported by the Arkansas Bioscience Institute under the project Development of an avian model for evaluation early enteric microbial colonization on the gastrointestinal tract and immune function. The authors thank the CONACyT for the doctoral grant number 270730.

Author details

Bruno Solis-Cruz[1], Daniel Hernandez-Patlan[1], Billy M. Hargis[2] and Guillermo Tellez[2*]

1 Faculty of Superior Studies Cuautitlán, Multidisciplinary Research Unit L5, National Autonomous University of Mexico, Cuautitlán Izcalli, Mexico State, Mexico

2 Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas, USA

*Address all correspondence to: gtellez@uark.edu

References

[1] Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathogens and Global Health, Taylor & Francis. 2015;**109**(7):309-318

[2] FAO. The FAO Action Plan on Antimicrobial Resistance 2016-2020. Rome, Italy; 2016

[3] OIE. The OIE Strategy on Antimicrobial Resistance and the Prudent Use of Antimicrobials. Paris, France; 2016

[4] WHO. Global Action Plan on Antimicrobial Resistance. Geneva, Switzerland; 2015

[5] WHO. WHO guidelines on use of medically important antimicrobials in food-producing animals: Web Annex A: Evidence base. 2017

[6] (EC) EC. Regulation No 1831/2003 of the European Parliament and Council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union. 2003;**268**:29-43

[7] US Food, Drug Administration D. New animal drugs and new animal drug combination products, administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI# 209. 2014

[8] Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrobial Agents and Chemotherapy. 2001;**45**(7):2054-2059

[9] Hao H, Cheng G, Iqbal Z, Ai X, Hussain HI, Huang L, et al. Benefits and risks of antimicrobial use in food-producing animals. Frontiers in Microbiology, Frontiers. 2014;**5**:288

[10] Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. Clinical microbiology reviews. The American Society for Microbiology. 2011;**24**(4):718-733

[11] Cervantes HM. Antibiotic-free poultry production: Is it sustainable? Journal of Applied Poultry Research, Poultry Science Association, Inc. 2015;**24**(1):91-97

[12] Siekkinen KM, Heikkilä J, Tammiranta N, Rosengren H. Measuring the costs of biosecurity on poultry farms: A case study in broiler production in Finland. Acta Veterinaria Scandinavica, BioMed Central. 2012;**54**(1):12

[13] FAO. Poultry Development Review. 2013. p. 120. Available from: http://www.fao.org/3/i3531e/i3531e00.htm

[14] Agyare C, Boamah VE, Zumbi CN, Osei FB. Antibiotic Use in Animal Production and its Effects on Bacterial Resistance. Rijeka, Croatia: IntechOpen; 2018

[15] Mehdi Y, Létourneau-Montminy MP, Gaucher ML, Chorfi Y, Suresh G, Rouissi T, et al. Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, Elsevier. 2018;**4**(2):170-178

[16] JETACAR. The use of antibiotics in food-producing animals: Antibiotic-resistant bacteria in animals and humans. In: Commonwealth Department of Health and Aged Care and the Commonwealth Department of Agriculture, Fisheries and Forestry, editor. Report of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR). Australia: Department of Health of the Australian Government; 1999

[17] Maron DF, Smith TJ, Nachman KE. Restrictions on antimicrobial use in food animal production: An international regulatory and economic survey. Globalization and Health, BioMed Central. 2013;**9**(1):48

[18] Castanon J. History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science, Oxford University Press. 2007;**86**(11):2466-2471

[19] Diarra MS, Malouin F. Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in Microbiology, Frontiers. 2014;**5**:282

[20] Cox LA Jr, Popken DA. Quantifying human health risks from virginiamycin used in chickens. Risk Analysis: An International Journal, Wiley Online Library. 2004;**24**(1):271-288

[21] Cox LA Jr, Popken DA. Quantifying potential human health impacts of animal antibiotic use: Enrofloxacin and macrolides in chickens. Risk Analysis: An International Journal, Wiley Online Library. 2006;**26**(1):135-146

[22] Sarangi NR, Babu L, Kumar A, Pradhan C, Pati P, Mishra J. Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. Veterinary World. 2016;**9**(3):313

[23] Bednarczyk M, Stadnicka K, Kozlowska I, Abiuso C, Tavaniello S, Dankowiakowska A, et al. Influence of different prebiotics and mode of their administration on broiler chicken performance. Animal, Cambridge University Press. 2016;**10**(8):1271-1279

[24] Ganguly S. Supplementation of prebiotics, probiotics and acids on immunity in poultry feed: A brief review. World's Poultry Science Journal, Cambridge University Press. 2013;**69**(3):639-648

[25] Pourabedin M, Zhao X. Prebiotics and gut microbiota in chickens. FEMS Microbiology Letters, Oxford University Press. 2015;**362**(15):fnv122

[26] Ricke S. Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. Poultry Science, Poultry Science Association, Inc. 2015;**94**(6):1411-1418

[27] Samal L, Behura N. Prebiotics: An emerging nutritional approach for improving gut health of livestock and poultry. Asian Journal of Animal and Veterinary. 2015;**10**:724-739

[28] Adhikari PA, Kim WK. Overview of prebiotics and probiotics: Focus on performance, gut health and immunity—A review. Annals of Animal Science, De Gruyter Open. 2017;**17**(4):949-966

[29] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology, Nature Publishing Group. 2017;**14**(8):491

[30] Ricke S. Impact of prebiotics on poultry production and food safety. The Yale Journal of Biology and Medicine. 2018;**91**(2):151-159

[31] Józefiak D, Kaczmarek S, Rutkowski A. A note on the effects of selected prebiotics on the performance and ileal microbiota of broiler chickens. Journal of Animal and Feed Sciences. 2008;**17**:392-397

[32] Slawinska A, Dunislawska A, Plowiec A, Radomska M, Lachmanska J, Siwek M, et al. Modulation of microbial

communities and mucosal gene expression in chicken intestines after galactooligosaccharides delivery In Ovo. PloS One, Public Library of Science. 2019;**14**(2):e0212318

[33] Siwek M, Slawinska A, Stadnicka K, Bogucka J, Dunislawska A, Bednarczyk M. Prebiotics and synbiotics-in ovo delivery for improved lifespan condition in chicken. BMC Veterinary Research, BioMed Central. 2018;**14**(1):402

[34] Piray A, Kermanshahi H, Tahmasbi A, Bahrampour J. Effects of cecal cultures and aspergillus meal prebiotic (Fermacto) on growth performance and organ weights of broiler chickens. International Journal of Poultry Science. 2007;**6**(5):340-344

[35] Li X, Qiang L, Xu C, et al. Effects of supplementation of fructooligosaccharide and/or *Bacillus subtilis* to diets on performance and on intestinal microflora in broilers. Archives Animal Breeding, Copernicus GmbH. 2008;**51**(1):64-70

[36] Sims M, Dawson K, Newman K, Spring P, Hoogell D. Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poultry Science, Oxford University Press. Oxford, UK. 2004;**83**(7):1148-1154

[37] Micciche AC, Foley SL, Pavlidis HO, McIntyre DR, Ricke SC. A review of prebiotics against salmonella in poultry: Current and future potential for microbiome research applications. Frontiers in Veterinary Science. Frontiers Media SA. 2018;**5**:1-11

[38] Bailey J, Blankenship L, Cox N. Effect of fructooligosaccharide on salmonella colonization of the chicken intestine. Poultry Science, Oxford University Press. Oxford, UK. 1991;**70**(12):2433-2438

[39] Fukata T, Sasai K, Miyamoto T, Baba E. Inhibitory effects of competitive exclusion and fructooligosaccharide, singly and in combination, on salmonella colonization of chicks. Journal of Food Protection, International Association for Food Protection. 1999;**62**(3):229-233

[40] Kim GB, Seo Y, Kim C, Paik I. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poultry Science. 2011;**90**:75-82

[41] Kim SA, Jang MJ, Kim SY, Yang Y, Pavlidis HO, Ricke SC. Potential for prebiotics as feed additives to limit foodborne campylobacter establishment in the poultry gastrointestinal tract. Frontiers in Microbiology, Frontiers Media SA. 2019:10

[42] Yadav AS, Kolluri G, Gopi M, Karthik K, Singh Y. Exploring alternatives to antibiotics as health promoting agents in poultry—A review. The Journal of Experimental Biology. 2016;**4**(3S-10.18006):3S

[43] Clavijo V, Flórez MJV. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. Poultry Science, Oxford University Press. 2018;**97**(3):1006

[44] Hajati H, Rezaei M. The application of prebiotics in poultry production. International Journal of Poultry Science. 2010;**9**(3):298-304

[45] Teng PY, Kim WK. Roles of prebiotics in intestinal ecosystem of broilers. Frontiers in Veterinary Science. Frontiers Media SA. 2018;**5**:1-8

[46] Shang Y, Kumar S, Oakley B, Kim WK. Chicken gut microbiota: Importance and detection technology. Frontiers in Veterinary Science, Frontiers. 2018;**5**:254

[47] Buclaw M. The use of inulin in poultry feeding: A review. Journal of Animal Physiology and Animal Nutrition, Wiley Online Library. 2016;**100**(6):1015-1022

[48] Gaggia F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, Elsevier. 2010;**141**:S15-S28

[49] Guillermo T, Andrea L, Juan DL, Xochitl HV, Billy MH, Todd C. Food-producing animals and their health in relation to human health. Microbial Ecology in Health and Disease, Taylor & Francis. 2015;**26**(1):25876

[50] Patterson J, Burkholder K. Application of prebiotics and probiotics in poultry production. Poultry Science, Oxford University Press. Oxford, UK. 2003;**82**(4):627-631

[51] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. Journal of Nutrition, Oxford University Press. 1995;**125**(6):1401-1412

[52] Xu Z, Hu C, Xia M, Zhan X, Wang M. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poultry Science, Oxford University Press. Oxford, UK. 2003;**82**(6):1030-1036

[53] Shang H, Hu T, Lu Y, Wu H. Effects of inulin on performance, egg quality, gut microflora and serum and yolk cholesterol in laying hens. British Poultry Science, Taylor & Francis. 2010;**51**(6):791-796

[54] Józefiak D, Rutkowski A, Martin S. Carbohydrate fermentation in the avian ceca: A review. Animal Feed Science and Technology, Elsevier. 2004;**113**(1-4):1-15

[55] Russell J. Another explanation for the toxicity of fermentation acids at low pH: Anion accumulation versus uncoupling. Journal of Applied Bacteriology, Wiley Online Library. 1992;**73**(5):363-370

[56] Van Immerseel F, Russell J, Flythe M, Gantois I, Timbermont L, Pasmans F, et al. The use of organic acids to combat salmonella in poultry: A mechanistic explanation of the efficacy. Avian Pathology, Taylor & Francis. 2006;**35**(3):182-188

[57] Tellez G, Dean C, Corrier D, Deloach J, Jaeger L, Hargis B. Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella enteritidis* organ invasion in Leghorn chicks. Poultry Science, Oxford University Press. Oxford, UK. 1993;**72**(4):636-642

[58] Van der Wielen PW, Biesterveld S, Notermans S, Hofstra H, Urlings BA, van Knapen F. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. The American Society for Microbiology. 2000;**66**(6):2536-2540

[59] Tellez G, Higgins S, Donoghue A, Hargis B. Digestive physiology and the role of microorganisms. Journal of Applied Poultry Research, Oxford University Press. Oxford, UK. 2006;**15**(1):136-144

[60] Willemsen L, Koetsier M, Van Deventer S, Van Tol E. Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E1 and E2 production by intestinal myofibroblasts. Gut, BMJ Publishing Group. 2003;**52**(10):1442-1447

[61] Huang Q, Wei Y, Lv Y, Wang Y, Hu T. Effect of dietary inulin supplements on growth performance and intestinal immunological parameters of broiler chickens.

Livestock Science, Elsevier. 2015;**180**:172-176

[62] Alloui MN, Szczurek W, 'Swikatkiewicz S. The usefulness of prebiotics and probiotics in modern poultry nutrition: A review. Annals of Animal Science, Versita 2013;13(1):17-32.

[63] Chen Y-S, Srionnual S, Onda T, Yanagida F. Effects of prebiotic oligosaccharides and trehalose on growth and production of bacteriocins by lactic acid bacteria. Letters in Applied Microbiology, Wiley Online Library. 2007;**45**(2):190-193

[64] Munoz M, Mosquera A, Almeciga-Diaz C, Melendez A, Sanchez O. Fructooligosaccharides metabolism and effect on bacteriocin production in lactobacillus strains isolated from ensiled corn and molasses. Anaerobe, Elsevier. 2012;**18**(3):321-330

[65] Ziprin RL, Corrier DE, Hinton A Jr, Beier RC, Spates GE, DeLoach JR, et al. Intracloacal *Salmonella typhimurium* infection of broiler chickens: Reduction of colonization with anaerobic organisms and dietary lactose. Avian Diseases, JSTOR. 1990:749-753

[66] Oyofo B, Droleskey R, Norman J, Mollenhauer H, Ziprin R, Corrier D, et al. Inhibition by mannose of in vitro colonization of chicken small intestine by *Salmonella typhimurium*. Poultry Science, Oxford University Press. Oxford, UK. 1989;**68**(10):1351-1356

[67] Oyofo B, DeLoach J, Corrier D, Norman J, Ziprin R, Mollenhauer H. Prevention of *Salmonella typhimurium* colonization of broilers with D-mannose. Poultry Science, Oxford University Press. Oxford, UK. 1989;**68**(10):1357-1360

[68] Spring P, Wenk C, Dawson K, Newman K. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poultry Science, Oxford University Press. Oxford, UK. 2000;**79**(2):205-211

[69] Chen TC. Effects of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. International Journal of Poultry Science. 2003;**2**(3):214-219

[70] Rehman H, Rosenkranz C, Böhm J, Zentek J. Dietary inulin affects the morphology but not the sodium-dependent glucose and glutamine transport in the jejunum of broilers. Poultry Science, Oxford University Press. Oxford, UK. 2007;**86**(1):118-122

[71] Johansson ME, Phillipson M, Petersson J, Velcich A, Holm L, Hansson GC. The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. Proceedings of the National Academy of Sciences. 2008;**105**(39):15064-15069

[72] Maiorka A, Silva A, Santin E, Dahlke F, Bruno L, Boleli I, et al. Effect of broiler breeder age and glutamine supplementation on the development of the intestinal mucosa of 7-day-old chicks. Brazilian Journal of Poultry Science, SciELO Brasil. 2016;**18**(1):17-22

[73] Bogucka J, Dankowiakowska A, Elminowska-Wenda G, Sobolewska A, Jankowski J, Szpinda M, et al. Performance and small intestine morphology and ultrastructure of male broilers injected in ovo with bioactive substances. Annals of Animal Science, De Gruyter Open. 2017;**17**(1):179-195

[74] De Los Santos FS, Donoghue A, Farnell M, Huff G, Huff W, Donoghue D. Gastrointestinal maturation is accelerated in Turkey poults supplemented with a mannan-oligosaccharide yeast extract (Alphamune). Poultry Science, Oxford University Press. Oxford, UK. 2007;**86**(5):921-930

[75] Ajuwon K. Toward a better understanding of mechanisms of probiotics and prebiotics action in poultry species. Journal of Applied Poultry Research, Oxford University Press. 2015;**25**(2):277-283

[76] Chapman M, Grahn M, Boyle M, Hutton M, Rogers J, Williams N. Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. Gut, BMJ Publishing Group. 1994;**35**(1):73-76

[77] De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell, Elsevier. 2014;**156**(1-2):84-96

[78] Matis G, Kulcsar A, Turowski V, Febel H, Neogrády Z, Huber K. Effects of oral butyrate application on insulin signaling in various tissues of chickens. Domestic Animal Endocrinology, Elsevier. 2015;**50**:26-31

[79] Yang Y, Iji P, Kocher A, Mikkelsen L, Choct M. Effects of dietary mannanoligosaccharide on growth performance, nutrient digestibility and gut development of broilers given different cereal-based diets. Journal of Animal Physiology and Animal Nutrition, Wiley Online Library. 2008;**92**(6):650-659

[80] Yun W, Lee D, Choi Y, Kim I, Cho J. Effects of supplementation of probiotics and prebiotics on growth performance, nutrient digestibility, organ weight, fecal microbiota, blood profile, and excreta noxious gas emissions in broilers. Journal of Applied Poultry Research, Oxford University Press. 2017;**26**(4):584-592

[81] Wang X, Lin X, Zhang L, Du Y, Bai X, Shu S. Effect of oligo-chitosan on broiler performance, small intestine structure and muscle mineral element concentration. Journal of the Chinese Cereals and Oils Association. 2005;**20**:83-88

[82] Li X, Piao X, Kim S, Liu P, Wang L, Shen Y, et al. Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poultry Science, Oxford University Press. Oxford, UK. 2007;**86**(6):1107-1114

[83] Swiatkiewicz S, Arczewska-Wlosek A. Prebiotic fructans and organic acids as feed additives improving mineral availability. World's Poultry Science Journal, Cambridge University Press. 2012;**68**(2):269-279

[84] Macfarlane GT, Steed H, Macfarlane S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. Journal of Applied Microbiology, Wiley Online Library. 2008;**104**(2):305-344

[85] Chen Y, Chen T. Mineral utilization in layers as influenced by dietary oligofructose and inulin. International Journal of Poultry Science, Asian Network for Scientific Information. 2004;**3**(7):442-445

[86] Scholz-Ahrens KE, Schrezenmeir J. Inulin and oligofructose and mineral metabolism: The evidence from animal trials. The Journal of Nutrition, Oxford University Press. 2007;**137**(11):2513S-2523S

[87] Ortiz L, Rodriguez M, Alzueta C, Rebolé A, Trevino J. Effect of inulin on growth performance, intestinal tract sizes, mineral retention and tibial bone mineralisation in broiler chickens. British Poultry Science, Taylor & Francis. 2009;**50**(3):325-332

[88] Swikatkiewicz S, Koreleski J, Arczewska-Wlosek A. Effect of prebiotic fructans and organic acids on mineral retention in laying hens. Acta Agriculturae Scandinavica Section A, Taylor & Francis. 2010;**60**(2):125-128

[89] Scholz-Ahrens KE, Schrezenmeir J. Inulin, oligofructose and mineral metabolism—Experimental data and mechanism. British Journal of Nutrition, Cambridge University Press. 2002;**87**(S2):S179-S186

[90] Whisner CM, Castillo LF. Prebiotics, bone and mineral metabolism. Calcified Tissue International, Springer. 2018;**102**(4):443-479

[91] Ghazalpour A, Cespedes I, Bennett BJ, Allayee H. Expanding role of gut microbiota in lipid metabolism. Current Opinion in Lipidology, NIH Public Access. 2016;**27**(2):141

[92] Wolever T, Spadafora PJ, Cunnane SC, Pencharz PB. Propionate inhibits incorporation of colonic [1, 2-13C] acetate into plasma lipids in humans. The American Journal of Clinical Nutrition, Oxford University Press. 1995;**61**(6):1241-1247

[93] Samanta A, Jayapal N, Senani S, Kolte A, Sridhar M. Prebiotic inulin: Useful dietary adjuncts to manipulate the livestock gut microflora. Brazilian Journal of Microbiology, SciELO Brasil. 2013;**44**(1):1-14

[94] Delzenne NM, Kok NN. Biochemical basis of oligofructose-induced hypolipidemia in animal models. The Journal of Nutrition, Oxford University Press. 1999;**129**(7):1467S-1470S

[95] Fiordaliso M, Kok N, Desager J-P, Goethals F, Deboyser D, Roberfroid M, et al. Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low-density lipoproteins of rats. Lipids, Wiley Online Library. 1995;**30**(2):163-167

[96] Kok N, Roberfroid M, Robert A, Delzenne N. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. British Journal of Nutrition, Cambridge University Press. 1996;**76**(6):881-890

[97] Lopetuso L, Giorgio M, Saviano A, Scaldaferri F, Gasbarrini A, Cammarota G. Bacteriocins and bacteriophages: Therapeutic weapons for gastrointestinal diseases? International Journal of Molecular Sciences, Multidisciplinary Digital Publishing Institute. 2019;**20**(1):183

[98] Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. Nutrients, Molecular Diversity Preservation International. 2011;**3**(10):858-876

[99] Vamanu E, Vamanu A. The influence of prebiotics on bacteriocin synthesis using the strain lactobacillus paracasei CMGB16. African Journal of Microbiology Research, Academic Journals. 2010;**4**(7):534-537

[100] Patterson JA. The Commensal Microbiota. Direct-Fed Microbials and Prebiotics for Animals. New York, USA: Springer; 2012. pp. 3-11

[101] Steed H, Macfarlane S. Mechanisms of prebiotic impact on health. In: Charalampopoulos D, Rastall RA, editors. Prebiotics and Probiotics Science and Technology. New York, NY: Springer; 2009. pp. 135-161

[102] Babu US, Sommers K, Harrison LM, Balan KV. Effects of fructooligosaccharide-inulin on salmonella-killing and inflammatory gene expression in chicken macrophages. Veterinary Immunology and Immunopathology, Elsevier. 2012;**149**(1-2):92-96