Chapter 07

Use of Probiotics and Prebiotics against Clostridial Diseases in Poultry

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ABSTRACT

Despite significant efforts in prevention and control, bacterial illnesses such as *Clostridium perfringens*-induced necrotic enteritis, continue to pose a serious threat to poultry producers in terms of economic losses and market limitations. Antibiotics have been utilized for growth promotion in addition to the prevention and management of such illnesses. Thus, these behaviours have been connected to the development and spreading of microorganisms that are resistant to antibiotics, posing a serious worldwide risk to people, animals, and the environment. Probiotics, prebiotics, and synbiotics are being used with great expectations. Primarily utilized to preserve the balance of the intestinal microbiota in cattle, they prove to be a successful strategy in combating infections that endanger both consumers and animals. This chapter provides a quick overview of the role that antimicrobial resistance plays in combating various bacterial infections, particularly clostridial infections, as well as the replacement of the antibiotics with probiotics, prebiotics, and synbiotics.

KEYWORDS		
Clostridium perfringens, Antibiotics, Probiotics, Prebiotics, Synbiotics, Illness, Microbiota	Received: 18-May-2024 Revised: 14-July-2024 Accepted: 17-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Finding high-quality feed that is both sustainable and reasonably priced is the largest obstacle to commercial chicken production. Despite this obstacle, commercial chick farming is one of the best ways to obtain animal protein (Iyayi, 2008). Compared to other animal sectors that produce food, the poultry business has grown at a higher rate. Parallel to the sharp rise in the world's output of chicken meat and eggs, there has been a rise in the volume of poultry goods traded (Windhorst, 2006).

The information at hand suggests that during the past few years, the chicken meat sector has experienced greater growth than the egg sector (Windhorst, 2006). In the chicken business, feed accounts for a large portion of the entire cost of production for producing meat and eggs. Under better conditions, five to six weeks can be required for broiler chickens to reach a weight of 2-3 kg. It is now difficult to prevent bacterial infections of the intestines without the need for medication in poultry production in Europe and America due to the ban on antibiotic growth boosters. Infection-related mortality is an important problem for the chicken sector.These illnesses cause decreased growth rates in poultry, which leads to financial losses. The primary instruments used to treat or prevent these diseases are antibiotics. The integrity of gastrointestinal tract (GIT) is vital to the health of birds because it acts as their intial defence against foreign invaders and facilitates the absorption of nutrients (Pan and Yu, 2014).

Numerous types of bacteria can be found in the GIT that make up the gut microbiota. These include *Lactobacillus, Clostridium, Ruminococcus, Salmonella enterica serovars*, Enterococcus spp., and *Escherichia coli*. They also maintain homeostasis and aid in the processing of nutrients. Mobile genetic elements like plasmids and transposons have the ability to horizontally transfer genes, hence introducing antimicrobial resistance genes (ARGs) into the gut microbiota. Antibiotics can alter the gut microbiota of chickens by lowering the number of harmful bacteria present, improving intestinal absorption of nutrients, and eventually improving growth characteristics (Diarra et al., 2007). Therefore, it's critical to comprehend how dietary habits affect the intestinal microbiota of chickens (Pan and Yu, 2014; Islam et al., 2019). Necrotizing enteritis (NE), a prevalent avian disease, is caused by the Gram-positive, spore-forming, obligate anaerobic bacterium C. perfringens and the global poultry business loses six billion dollars annually as a result of it(McDevitt et al., 2006). This bacterium is environmental pathogen and normally present in the digestive tracts of humans and animals, and when body undergo stress, it also poses a threat to food safety (Collier et al., 2003). Subclinical NE causes poor feed conversion ratios and decreased weight growth, which in turn causes production losses (Stutz and Lawton, 1984). When *C. perfringens* causes intestinal injury, the germs have access to the bile duct and bloodstream, which destroys other organs in birds (Timbermont et al., 2011). Common antibiotics used to prevent NE in poultry include avilamycin and bacitracin methylene disalicylate. Controlling this infection has therefore become extremely important due to the AMU limits in poultry, not only for the gut health of the birds but also for the sake of food safety (McDevitt et al., 2006). Unfortunately, extended and irrational use of antibiotics in animals can ultimately select for strains or species resistant to the drugs (Aarestrup, 1999). Because genes encoding this resistance can spread to other bacteria that were previously vulnerable, the health of humans and animals may be in danger (Montagne et al., 2003). Consequently, several countries like European Union in January 2000 have limited or completely forbidden (Sweden, January 1986) the use of in-feed medications as growth promoters for animals (Montagne et al., 2003)**.** To enhance performance and control illness, Antibiotic growth promotants (Nagpal et al.) are added to the diets of food animals. The use of enzymes, probiotics, prebiotics, symbiotic products, and even nutrition to improve chicken gut health and prevent or reduce production losses due to enteric illnesses has been investigated by researchers as a potential more effective feed additive than AGP.

Probiotics

The Greek terms "pro" and "bios" give rise to the English word "probiotic," which means "for life." It is likely that Mechnikov, who observed that the initial concept of probiotics was introduced in 1907, suggesting that bacteria could positively impact the normal microflora in the intestinal tract (Miecznikow, 1907). Ferdinand Vergin is credited with coining the word "probiotic" in his 1954 study "Anti- und Probiotika," which compared the beneficial effects ("probiotica") of specific bacteria with the harmful effects of antibiotics and other antimicrobial drugs on the gut microbiota (Vergin, 1954).

Probiotics are defined as “live isolates of strictly specified microbes which, when supplied in sufficient quantities, confer beneficial health effects on the host” in the definition that was developed in 2002 by experts from the FAO and WHO working groups (Joint, 2002). In 2013, ISAPP upheld the idea. A recipe or product is only considered "probiotic" if it satisfies certain tight requirements. The three most crucial of these requirements are: a suitable number of functional cells; a positive impact on the host's health (which may include growth promotion); and a positive impact on the alimentary tract's performance. The effectiveness of probiotic supplements is determined by various factors. It is therefore essential to select the appropriate bacterial strains and utilize the appropriate dosage.Probiotics are beneficial to health and promote growth, that's why they are frequently utilized in animal feed, particularly for chickens and pigs. These kinds of formulas could include one or more carefully selected bacterial isolates, and their administration methods may include powder, suspension, capsules, pellets, gels, or pastes, contingent on the host animals' age and species. These are added to feed and premixes or utilized as a direct peros supplement on a periodic or continuous basis. When probiotic cultures are added to feed, they have to fulfil certain requirements (Markowiak and Śliżewska, 2018).

Probiotic Microorganisms

Products that contain probiotics may contain one or more particular bacterial isolates. In the EU, the majority of microbes utilized as feed additives are bacteria. *Pediococcus, Lactobacillus, Enterococcus, Bacillus, and Streptococcus* are the genera from which they are usually Gram-positive bacteria. Probiotics include certain yeast strains from Kluyveromyces species and Saccharomyces cerevisiae. Bacteria from the genera *Lactobacillus and Enterococcus,* which are normally found in levels of 107–108 and 105–106 CFU/g, respectively, make up the natural microflora of the animal gastrointestinal tract. However, the digestive system typically does not include yeast or bacteria from the *Bacillus* genus. Most of the microbes listed above ought to be potentially harmless to the host. Some of them, though, could be problematic. For example, antibiotic resistance may spread due to bacteria belonging to the *Enterococcus* genus and certain strains of *B. cereus* could create the endotoxins and emetic toxins (Anadón et al., 2006).

Mode of Action of Probiotics

Diseases may arise based on how the host and microorganism interact (Garcia et al., 2010). The exact mode of action of SFPs remains unclear. Nonetheless, it has been found that SFPs function via the same mechanism as regular probiotic microbes (Cartman et al., 2008). Probiotics' working principle in poultry has been described in a variety of ways (Figure no 1). The first method was termed as "competitive exclusion."; it works by preventing harmful germs from colonizing sites of adhesion through competition for them (Chichlowski et al., 2007). Beneficial bacteria's adherence allowed for this competitive exclusion to occur (Chichlowski et al., 2007). The capacity of the SFP strain to cling to the intestinal wall is necessary for the continuation of this mechanism of action (Fuller, 1989). The metabolites produced by gut bacteria compete with pathogenic substances for adherence in the epithelium of the intestine and regulate their growth. These metabolites include hydrogen peroxides, bacteriocins, and short-chain organic acids. (Dankowiakowska et al., 2013) assist in adhesion to the mucosal layer of the intestine (Buck et al., 2005). The newborn's digestive tract is sterile, and germs from the surroundings begin to infiltrate the gut before its organs can manufacture antibodies. As a result, using probiotics helps to naturally inhibit the microorganisms that cause sickness because of their capacity to adhere to the gut mucosa (Dankowiakowska et al., 2013). Probiotics have been shown to have a second mode of action that involves regulating dysbiosis. Enteric dysbiosis has the potential to modify host-microbe interactions, resulting in medical disorders (Byndloss and Bäumler, 2018; Plaza-Díaz et al., 2018). Probiotics may be able to treat dysbiosis and balance out the disturbed or unbalanced microflora, according to recent research (Vieira et al., 2016; Mendes et al., 2018). Probiotics' antagonistic action enabled the third mechanism to function. A class of tiny antimicrobial compounds produced by probiotics (called bacteriocins, mucins, and defensins) prevents infections from colonizing (Khan and Naz, 2013). The most significant advantage of probiotics is thought to be their ability to modify GI immunity, which is known to be the fourth mode of action. Numerous investigations have demonstrated that probiotics stimulate the production of anti-inflammatory mediators, including transforming growth factor beta (TGF-β), thymic stromal lymphopoietin (TSLP), and interleukin-10 (IL-10). Additionally, concurrently reduces the generation of pro-inflammatory cytokines such as TNF-α and interleukin 8 (IL-8) (Georgieva et al., 2015). The ways in which probiotics work are:

1. Competitive exclusion of harmful bacteria by probiotics.
2. Probiotics restore dysbiosis resulting from any cause.
3. Probiotics generate bactericidal chemicals that lyse infections, whereas probiotic surface receptors deactivate and disrupt microbes.
4. When intestinal cells come into contact with pathogens, they release pro-inflammatory substances such as TNF-α, interleukin-8, and interleukin- 12. By producing more anti-inflammatory mediators like TGF-β and TSLP, probiotics reduce the synthesis of pro-inflammatory chemicals and help transform premature dendritic cells into regulatory dendritic cells. TGF-β, or transforming growth factor-β; TSLP, lymphopoietin in stroma of thymus (Khalid et al., 2022).

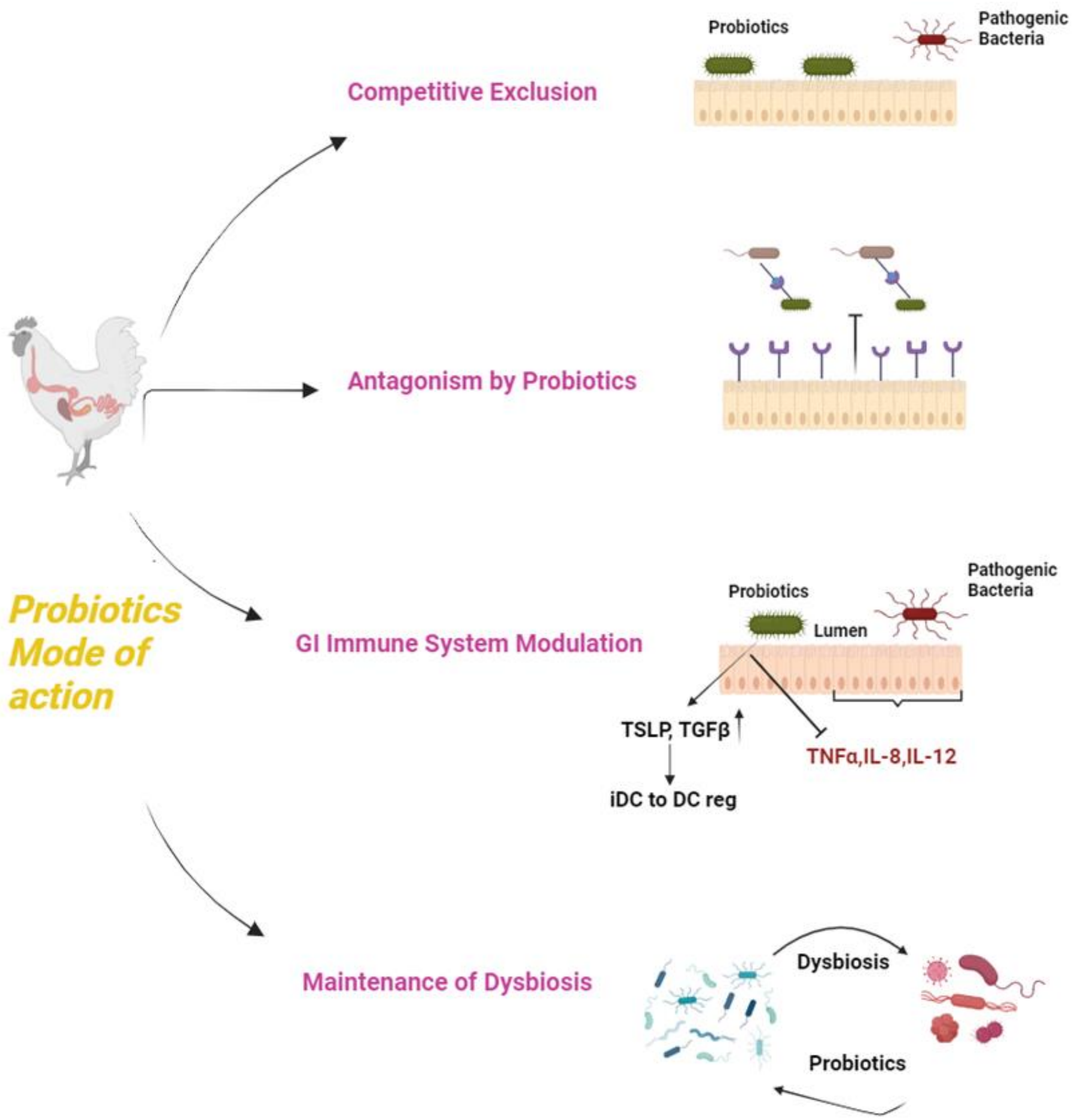

Fig. 1: Modes of action of probiotics.

Prebiotics

Prebiotics are another type of natural feed additive used in addition to probiotics. The addition of lactic bacteria to the human gut microbiota after carbohydrate eating was originally documented by Rettger and Cheplin in 1921.(Rettger, 1921). The notion of prebiotics was initially introduced in 1995 (Gibson and Roberfroid, 1995). The definition used nowadays was coined in December 2016 by ISAPP. Prebiotics, by definition, are a class of substances that function outside of the digestive tract. These substances include molecules other than carbohydrates, like polyphenols and polyunsaturated fatty acids that have been changed into fatty acids that are conjugated that are comparable. They may now be considered for other groups, such as nourishment for animals, and are no longer just for human consumption, which is another significant development. However, standards regarding the specific processes of microflora manipulation and the requirement of proven positive impacts on host health have been upheld (Gibson et al., 2017). Numerous substances, including cellulose, xylanes, and pectins, promote the growth of diverse gut microbes. Prebiotics should stimulate specific metabolic pathways rather than being heavily metabolized in order to promote the host's ecosystem's health. Benefits from using indigestible oligosaccharides, like fructans and galactans, have been well-documented (Rastall and Gibson, 2015).

Prebiotic Substances

Prebiotic compounds include proteins, lipids, peptides, and carbohydrates that are not absorbed (oligosaccharides and polysaccharides). Fruit, cereals, and legumes are organic sources of prebiotic bacteria. However, commercial chemical and enzymatic processes are used to produce numerous comparable compounds (Śliżewska et al., 2013). Several prebiotics are often used, including FOS, oligofructose, gluco-oligosaccharides, glico-oligosaccharides, lactulose, lactitol, malto-oligosaccharides, xylo-oligosaccharides, stachyose, and raffinose. (Monsan and Paul, 1995; Orban et al., 1997; Patterson et al., 1997; Collins and Gibson, 1999; Patterson and Burkholder, 2003). Upon arrival in the large intestine, those materials serve as nourishing substrates for healthy intestinal microorganisms (Grajek et al., 2005). These are the classes into which prebiotics fall based on characteristics that indicate a positive impact on the health of host: not broken down (or only partially broken down), not absorbed in the small intestine, inadequately fermented by bacteria in the mouth, well-fermented by gut microbes that seem to be beneficial, and poorly fermented by possible intestinal microbes (Markowiak and Śliżewska, 2018). Lactol, lactulose, cereal fiber, isomalto-oligosaccharides (IMO), xylo-oligosaccharides (XOS), FOS, and GOS are the prebiotics that are most frequently used in animal feeding.Determining the right dosage is crucial when creating prebiotic formula compositions. Flatulence and diarrhoea may result from taking too many prebiotics. However, a big benefit of such kinds of formulae is that they have no known side effects and can be used long-term as a preventative measure (Olveira and González-Molero, 2016; Markowiak and Śliżewska, 2018).

Mode of Action of Prebiotics

As was previously indicated, there are many different kinds of prebiotics; in this case, we will only talk about fructans and how they work. Fructans, which are usually produced by microbes or hydrolyzed from polysaccharides, have been added to broiler diets recently. There are three different groups of fructans: the branching group, the levan group, and the inulin group. Initially, according to DP (degrees of polymerization), the inulin group, commonly referred to as fructooligosaccharides (FOS), can be categorized into the following groups: The DP of inulin, which is typically derived from chicory roots (Cichorium intybus L.), ranges from 3 to 60. Oligofructose (OF), on the other hand, has a DP of 2 to 10 and can be produced by partial breakdown of inulin, lactose or sucrose conversion via catalysis (Ritsema and Smeekens, 2003; Rossi et al., 2005). Oligosaccharides containing β-2,1 fructosyl-fructose linkage and a glucose terminal unit are found in plants, which makes up the majority of the inulin group. Second, there is another group of fructans called the levan group that are mainly linked by β-2,6 fructosyl fructose bonds. Last but not least, fructans, which are members of the branching group, have fair proportions of both β-2,1 fructosyl-fructose and β-2,6 fructosyl fructose linkages (Zhao et al., 2013). Because of their β-glycosidic link, fructans are protected from being broken down by the digestive enzymes of fowl, which increases the amount of good bacteria (like *Bifidobacteria and Lactobacilli*) and decreases the amount of bad bacteria (like *E. coli and C. pefringens*) in the broiler's intestine (Xu et al., 2003; Kim et al., 2011; Ricke, 2015). Long-chain fructans can cross the small intestine and ferment in the distal sections of the intestine, despite their slow breakdown in the animal gut. As a result, the inulin group with higher DP may not have a substantial impact on the jejunum's microbiota, rather, it could change the composition of microorganisms and raise the levels of lactic acid or SCFA in broiler ceca. (Rehman et al., 2008). The effects of fructans on intestinal microbiota are demonstrated by the increased levels of two important beneficial microbes, *Bifidobacteria* and *Lactobacillus*, in the feed of broilers and hens that contain fructans (Rada et al., 2001; Xu et al., 2003; Rebolé et al., 2010; Zhao et al., 2013). In addition to producing extracellular enzymes to break down fructooligosaccharides (FOS), *Lactobacillus* and *Bifidobacteria* also acted as competitors with other gut microbe species, stop harmful microbes from multiplication (Rossi et al., 2005). For example, broilers given FOS had lower levels of *Campylobacter* titers in the large intestine and ceca (Zhao et al., 2013). In the ileocecal junction or ceca of broilers, a decrease in *C. perfringens* titers was seen irrespective of the addition of long- or short-chain FOS (Xu et al., 2003; Biggs et al., 2007; Zhao et al., 2013). In contrast, the colonization of cecal *C. perfringens* and *Salmonella typhimurium* in *E. coli* and FOS alone or in combination with the products that cause the removal of harmful pathogens, reduced the number of chickens challenged with *Salmonella*(Yang et al., 2008; Telg and Caldwell, 2009). Additionally, diets ranging in FOS concentration from 0.25 to 1% may lessen the amount of *Salmonella* and *E. Coli* in broiler cecum (Xu et al., 2003; Li et al., 2008; Kim et al., 2011; Zhao et al., 2013). Lactic acid and cecal SCFA may have contributed to the decrease in these harmful bacteria. Similar to in vitro findings, broilers fed inulin had much higher levels of lactic acid and cecal butyric acid (Rehman et al., 2008; Rebolé et al., 2010). Butyrate is the primary short-chain fatty acid that is metabolized by epithelial cells in the intestine, serving as a vital fuel for the growth of intestinal epithelium (Topping and Clifton, 2001). It has been proposed that improved mucosal structure is related to increased butyric acid levels. According to earlier research, In the ceca, FOS enhanced the villus to crypt depth ratio.and the height of microvillus in the ileum and jejunum. Yet, fructan additions, might have detrimental effects on broilers (Xu et al., 2003; Rebolé et al., 2010). The intestine's bacteria may ferment food too quickly, producing an excess of SCFA that damages intestinal mucosal barriers and increases intestinal permeability. This can lead to diarrhoea, poor development, and invasion by pathogens (Wu et al., 1999; Wu et al., 2005). Yet, High fructan additions, might have detrimental effects on broilers. The intestine's bacteria may ferment food too quickly, producing an excess of SCFA that damages intestinal mucosal barriers and increases intestinal permeability. This can lead to diarrhoea, poor development, and invasion by pathogens (Wu et al., 1999; Wu et al., 2005). Through these two main pathways, fructans enhanced the immunological responses of the immune system of body and lymphoid tissue present in gut. First of all, fructans can raise Bifidobacteria levels, which may alter cytokine or antibody production. Second, after leukocytes' receptors react to metabolites of fructans, like SCFA, leukocytes may become activated. All things taken into account, fructans may affect intestinal microbes, intestinal SCFA levels, mucosal architecture, and immunological responses. In the intestinal microbiota of broilers, there will be an increase in *Lactobacillus*, *Bifidobacteria* and there will be a decrease in pathogens like *E. coli*, *C. perfringens,* and *Salmonella (*Figure 2). There will be immune system modulation due to *lactobacillus* and *bifidobacteria* production. Fermentation of fructans will give rise to (SCFA's) short-chain fatty acids, which will cause an increase in MUC gene expression, an increase in microvillus height, decrease in depth of crypt, increase in height of Villus: crypt depth and these changes will also inhibit the pathogen colonization (Teng and Kim, 2018).

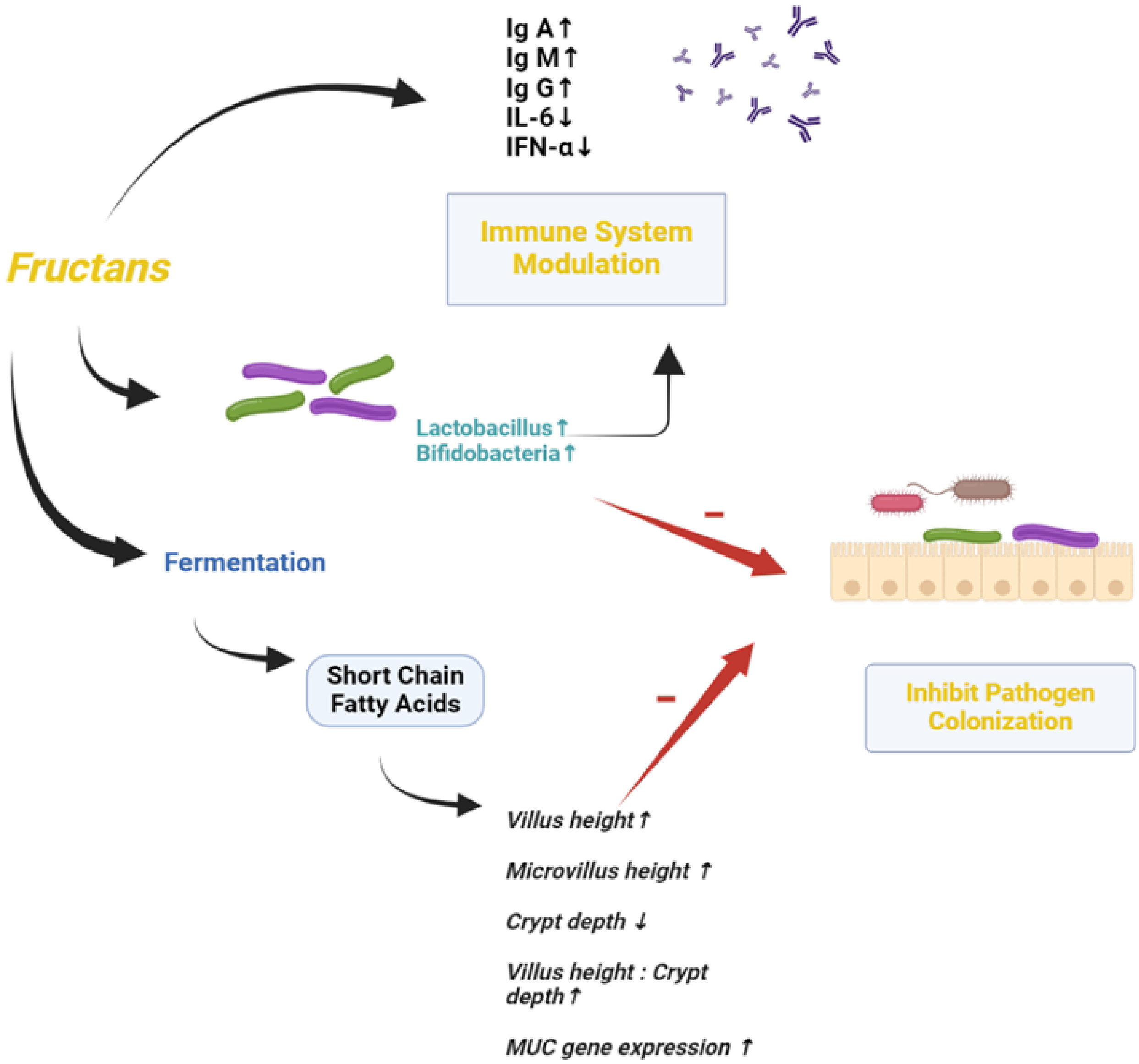

Fig. 2: Mode of action of prebiotics.

Synbiotics

In animal nutrition, the combination of probiotics and prebiotic are also utilized. When they used the term "synbiotic" in 1995, Gibson and Roberfroid described it as " a probiotic-prebiotic blend that enhances the host's ability to survive and introduce nutritional supplements containing live microorganisms into the gastrointestinal system,by enhancing host welfare by the targeted stimulation of one or a small development of numerous microorganisms that promote health and metabolism" (Gibson and Roberfroid, 1995). The term "synbiotic" should only be applied to products where a prebiotic component preferentially benefits a probiotic bacterium since it suggests synergy (Cencic and Chingwaru, 2010). Enhancing the survival of probiotic bacteria in the gastrointestinal system is the main goal of that kind of combination. Synbiotics were developed to address potential obstacles to probiotic survival in the gastrointestinal system. They possess both probiotic and prebiotic qualities (Rioux et al., 2005). Probiotics operate as a barrier to protect the gastrointestinal tract and have a favorable impact on intestinal balance. Conversely, probiotic bacteria receive their energy and nourishment from prebiotics (Gibson and Roberfroid, 1995). In light of this, a product containing both ingredients in a suitable ratio ought to have a greater impact than just the probiotic or prebiotic acting individually (Panesar et al., 2009).

Synbiotics in Use

Probiotic bacteria and prebiotic materials most frequently utilized in animal feeding were covered in earlier sections. The most widely used combination in synbiotic products appears to be FOS with bacteria from the Lactobacillus or Bifidobacterium genera (Markowiak and Śliżewska, 2018).

Synbiotics for Animals

Conventional DNA-based molecular tools have made significant strides in recent years in terms of both research and application, giving microbiologists new and unparalleled capabilities for characterizing and comprehending microbial populations (Pontes et al., 2007). Microbiologists can examine a more comprehensive picture of environmental microbiological communities through metagenomic investigations, which include clone library construction and screening as well as the isolation of entire microbial community genomes. This helps them to better understand the interactions between microbes and their surroundings (Singh et al., 2008). Metagenomics appears to be a viable method for evaluating the synbiotic impact of animal gut microbiota.

It has been confirmed that FOS and *Lactobacillus paracasei* bacteria have a synergistic effect on piglets' gut microbiome (Nemcova et al., 1999). An increase in the total count of obligate aerobes and as well as an increase in the quantity of beneficial *Lactobacillus and Bifidobacterium* genus bacteria has been observed in the animal group by researches. Simultaneously, the number of bacteria belonging to the species *Clostridium, E. coli,* and *Enterobacteriaceae* reduced in the stool of the piglets under study (Nemcova et al., 1999). The impact of the product that contain *Enterococcus faecium* bacteria as probiotic and fructooligosaccharides (FOS) as prebiotic, and immune-modulating compounds derived from marine algae (ficophytic chemicals) on the health of broiler chickens was investigated by (Awad et al., 2009). Five weeks of breeding 600 broiler chickens were examined. FCR, BW gain on daily basis, and carcass ratio, all showed a discernible increase in comparison to control animals (Awad et al., 2009). In summary, researchers agree that applying probiotics and prebiotics separately is less beneficial than utilizing synbiotic products (Biggs et al., 2007; Awad et al., 2009; Revolledo et al., 2009).

Conclusion

Prebiotics, probiotics help in maintaining a healthy gut microbiota in poultry. Prebiotics function as a dietary source for beneficial bacteria and probiotics introduce beneficial bacteria in the gut. To strengthen poultry's defenses against a variety of intestinal ailments, probiotics and prebiotics are recommended. They cause the activation of cytokines, other immune mediators and induce the synthesis of immunoglobins, thus enhancing the competitive exclusions of enteric bacteria by occupying attachment sites in the gut lining, thereby reducing the colonization and proliferation of enteric pathogens. Prebiotics and probiotics offer a natural and sustainable approach to disease prevention without contributing to the development of antibiotic-resistance strains. Prebiotics and probiotics can improve the feed efficiency, nutrient utilization and growth performance in poultry. They contribute to the sustainability of poultry production summary. In summary, a comprehensive strategy for boosting gut health, boosting immunity, lowering pathogen load, and boosting overall performance while addressing antibiotic resistance problems is provided by the use of prebiotics and probiotics in chicken production.

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