



Review article

## Strategies to attack pathogenic avian microorganisms: From probiotics to postbiotics

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### Abstract

To reduce the growing risk of antimicrobial resistance, there is an increasing demand to substitute synthetic antimicrobial growth promoters in animal production with safer natural chemicals or biological alternatives. Therefore, this chapter will focus on the use of probiotics, prebiotics, synbiotics, and postbiotics in poultry production. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Prebiotics are considered a substrate that is selectively utilized by host microorganisms, conferring a health benefit. They are thought to be hydrolyzed and then used by the gastrointestinal tract bacteria found in different parts of the avian gastrointestinal tract because they have been described as indigestible by the host. There are five categories of prebiotics: fructans, galactooligosaccharides, starch and glucose-derived oligosaccharides, other oligosaccharides, and non-carbohydrate or miscellaneous like cocoa-derived flavanols, polyphenolics, fatty acids, herbs, and other supplements. The most often used prebiotics in poultry include fructo-oligosaccharide, mannan-oligosaccharides, and galacto-oligosaccharides. A synbiotic is a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms, conferring a beneficial effect. There are complementary and synergistic synbiotics. In chickens, synbiotics can be supplemented in feed or water or injected *in ovo* to expedite colonization of the gut by beneficial bacteria. Finally, postbiotics are considered inactivated microbial cells or cell components, with or without their metabolites, that provide health benefits. Many existing postbiotics include inanimate strains belonging to established probiotic taxa within some genera of the family *Lactobacillaceae* or the genus *Bifidobacterium*. Postbiotics are composed of food-grade microorganisms or released after cell lysis in complex microbial cultures, food, or the intestinal lumen. All these products help support a healthy gut and immune system in poultry.

**Keywords:** Probiotic, Prebiotic, Synbiotic, Postbiotic, Poultry

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### Introduction

Microbiota is known as the entire population of commensal, synbiotic, and pathogenic microorganisms (consisting of viruses, protists, fungi, bacteria, and archaea) that live on or inside complex multicellular organisms (including humans, animals, and plants). Over the past ten years, our understanding of the microbiota's significance for both human and animal health has significantly increased (Hou et al., 2022). The gastrointestinal tract (GIT)

microbiota of broiler chickens has been shown to be important for the host's health because it influences the immune system, the physiology of the GIT, and the productivity of the animal. Through bacteriostatic and bactericidal chemical synthesis, as well as competitive exclusion, the microbiota of broilers contributes to the reduction and prevention of enteric pathogen colonization. Therefore, inflammation, leaky gut, and other gut-related conditions can be brought on by an unbalanced microbiota. In this case,

maintaining gut health is essential to guaranteeing poultry development and wellness at their best (Shang et al., 2018). Numerous factors, including age, feeding, genetics, and, most notably, the usage of antibiotics, influence the microbiota of broilers (Shang et al., 2018). The broad-spectrum nature of the most widely prescribed antibiotics suggests antimicrobial medication kills non-targeted and typically helpful microorganisms, causing significant collateral damage to the host's microbiota. This adverse consequence frequently results in dysbiosis, which encourages the growth of bacteria resistant to antibiotics and may even cause the horizontal transfer of the resistance genes (Roth et al., 2019).

Poultry producers are continuing looking for a viable and safe alternative to in-feed medications, especially producers who have modified their conventional production practices or moved toward Raised Without Antibiotics (RWA), No Antibiotics Ever (NAE), No Human Antibiotics (NHA), No Medically Important Antibiotics (NMIA), No Critical Important Antibiotic (NCIA), or No Growth-Promoting Antibiotic (NGPA) production systems (Calvo and Meltzer-Warren, 2020). These trends started in response to concerns raised by the World Health Organization (WHO) on the emergence of multi-antimicrobial resistant human pathogens, and those practices increase production costs with questionable effects on meat, egg, or dairy consumer demand. In particular, RWA production has been accelerated by demands made and pressure applied by consumer activists' groups on regulatory agencies, grocery retailers, fast-food outlets, and restaurant chains. In turn, large buyers of chicken products have demanded that broiler companies supplying them comply with these demands. In some instances, marketing campaigns initiated by fast-food outlets, restaurant chains, or poultry companies themselves have resulted in the increased production of RWA chickens. As the RWA segment of poultry production continues to grow, veterinarians and production managers must practice more diverse management practices to control diseases (Singer et al., 2019; Cervantes, 2023).

Many countries have banned the use of antibiotics in animal feed as antimicrobial growth promoters (AGP) due to concerns about antimicrobial resistance. In other countries,

antibiotics that are medically important for humans have been voluntarily or regulated to be removed or significantly reduced in poultry production. There are also places where veterinarians prescribe antibiotics, and sick poultry can still be treated if a veterinarian deems it necessary (Rahman et al., 2022). In response, several alternatives to antibiotics, such as probiotics, prebiotics, synbiotics, postbiotics, phytochemical substances, organic acids, and bacteriophages, have been developed, tested, evaluated, and used for poultry production at an increasing frequency (Abd El-Hack et al., 2022; Dong et al., 2024). Some of those have been referred to in recent years as "Eubiotics," a broad term that is derived from the Greek word "Eubiosis" and denotes an ideal balance of the microbiota in the gastrointestinal system. The major goal of employing these eubiotics is to preserve intestinal eubiosis, which will enhance farm animals' health and productivity (Barragry, 2020). In this review, we give an update on the use of probiotics, prebiotics, synbiotics, and postbiotics in poultry production.

### **Probiotics**

In 2001, the WHO and the Food and Agricultural Organization of the United Nations (FAO) held an expert consultation and defined the word "probiotic." The next year, Guidelines for the Evaluation of Probiotics in Foods were released by FAO/WHO (FAO and WHO, 2006). An Expert Working Group that convened before the first meeting of the International Scientific Association for Probiotics and Prebiotics (ISAPP) drafted these guidelines. An Expert Panel was gathered in 2013 by ISAPP to examine the word "probiotic" and related publications. They retain the FAO/WHO definition for probiotics, with a minor grammatical correction as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Probiotics are categorized by strain, genus, and species.

Given that distinct strains of the same species may have diverse health benefits, strain classification is crucial. Another factor to consider is dosage; a probiotic taken at one level may not always be more beneficial than one taken at a lower dose. For a dose to be beneficial, it must correspond with the level seen in an efficacy study (Hill et al., 2014).

The processes behind the health advantages of

probiotics are being studied and may even be understood in some situations. While a significant health benefit has been shown, mechanisms remain unclear and difficult to validate in humans and animals (ISAPP, 2020). The suggested mechanisms by which probiotics exert their action include competitive exclusion of pathogens for adhesion sites, production of inhibitory substances, improvement of the intestinal mucosal barrier, gut immunomodulation, and neurotransmitter synthesis. Experts also believe that several pathways might work together to produce a health benefit (Mathipa and Thantsha, 2017; Latif et al., 2023). A prevalent misperception is that probiotics cannot function properly unless they modify the intestinal biota. Despite their established health advantages, probiotics have not been demonstrated to take up permanent residence in the gut (ISAPP, 2020).

Many different fungi, protozoa, and bacteria have been investigated for their probiotic properties and used in field trials, but only a few number have become commercially available (Jeni et al., 2021). For an organism to be considered a probiotic, it must meet a series of requirements, such as having its *in vitro* characterization, which implies knowing the phenotypic and genotypic stability and the utilization patterns of carbohydrates and proteins. In addition, resistance to gastric acidity, resistance to bile, adhesion to the intestinal epithelium, and resistance to lysozyme (optional) are considered. Other factors that should be taken into account are the ability to use prebiotics (optional) and the existence of *in vivo* and *in vitro* trials that demonstrate the claimed probiotic effects. Likewise, they must be generally regarded as safe (Generally Recognized as Safe, GRAS) and not present resistance to antibiotics or determinants of pathogenicity (Blajman et al., 2015). Because some cell viability is lost during the production of probiotic cultures and over the product's shelf life, manufacturers typically overdose their products with live cells above the recommended dosage to ensure enough live cells at the end. If probiotics are administered over an extended period in future efficacy trials, it could be worthwhile to consider the build-up of inactive cells during the probiotic's shelf life (Vinderola et al., 2022).

### Probiotic sources

Probiotics are typically made from a variety of live microorganisms, bacteria and/or fungi (Markowiak and Śliżewska, 2018). Live yeast and bacteria are similar in that they both provide health benefits to poultry. The difference lies in how each organism achieves those health benefits. Probiotic bacteria isolates are intended to produce compounds or enzymes such as phytases, cellulases, proteases, or xylanases (Krysiak et al., 2021). Most probiotic bacteria, including lactic acid bacteria (LAB), *Bifidobacterium* spp, and *Bacillus* spp, are non-spore-forming, anaerobic, Gram-positive cocci or rods that ferment carbohydrates primarily to lactic acid. There is a core group made up of four genera: *Streptococcus*, *Leuconostoc*, *Pediococcus*, and *Lactobacillus*. The following genera now make up the remaining group after recent taxonomic changes: *Aerococcus*, *Alloiococcus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella*. Their safe metabolic activity during food growth, which uses available sugar to produce organic acids and other metabolites, is primarily linked to their significance. Their widespread presence in food and their enduring use led to their automatic designation as GRAS for ingestion by humans (Bintsis, 2018). Furthermore, recently, based on this polyphasic approach, the genus *Lactobacillus* was reclassified into 25 genera, including host-adapted organisms that have been referred to as the *Lactobacillus delbrueckii* group, *Paralactobacillus* and 23 novel genera. The names proposed for the novel 23 genera are *Holzapfelia*, *Amylolactobacillus*, *Bombilactobacillus*, *Companilactobacillus*, *Lapidilactobacillus*, *Agrilactobacillus*, *Schleiferilactobacillus*, *Loigolactobacillus*, *Lactica-seibacillus*, *Latilactobacillus*, *Dellaglioia*, *Liquorilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Furfurilactobacillus*, *Paucilactobacillus*, *Limosilactobacillus*, *Fructilactobacillus*, *Acetilactobacillus*, *Apilactobacillus*, *Levilactobacillus*, *Secundilactobacillus* and *Lentilactobacillus* (Zheng et al., 2020). In addition to requiring familiarity with 23 new genus names, the new taxonomy of lactobacilli offers significant new potential for scientific research and regulatory licensing of these organisms. Initially, the *Lactobacillaceae* family offers a strong framework for the description of new genera, which facilitates the description of new species. Furthermore, the present taxonomy significantly improves the

resolution of genus-level sequencing methods (such as 16S rRNA-based metagenomics) for determining the makeup and role of microbial communities. Additionally, the present taxonomy makes it much easier to formulate theories that relate lactobacilli phylogeny to metabolism and ecology (Qiao et al., 2022). There is a web-based application (<http://lactobacillus.ualberta.ca/>) that makes it very simple to find the new names for all *Lactobacillus* species.

On the other hand, taxonomically classified as members of the *Bifidobacteriaceae* family, bifidobacteria are Gram-positive, non-motile, non-spore-forming, anaerobic, saccharolytic microorganisms with a Y-shaped or 'bifid' morphology, and a high G+C DNA content. Many members of the genus *Bifidobacterium* possess, relative to their genome size, one of the largest genetic repertoires for glycosyl hydrolases and carbohydrate uptake systems involved in the degradation and internalization of plant- and host-derived glycans. This genetic arsenal endows bifidobacteria with powerful and flexible metabolic strategies to compete with other members of the gut microbiota to ensure their fitness in the intestinal environment (Alessandri et al., 2021).

Probiotics, particularly those belonging to the *Bacillus* species, are alternative probiotic candidates because of their ability to form spores, giving them several advantages over traditional probiotics. The various mechanisms that these organisms possess to produce probiotic effects have been linked to their success. These mechanisms include competitive exclusion of common poultry pathogens, improved digestion and absorption through the production of exogenous enzymes, improved intestinal morphology, immunomodulation, and reduction of toxic compounds like ammonia and aflatoxins. These benefits lessen illness and death, increase feed efficiency by 5%, improve health, and support environmentally sustainable poultry farming. *Bacillus* species can thrive in the tough circumstances of the gastrointestinal tract and are easily segregated from their surroundings. Aside from these crucial factors, the main benefit of using *Bacilli* as feed probiotics is their robustness in terms of industrial production due to their high-density spore production, which can produce more than  $1 \times 10^{11}$  spores/mL. In addition, spores can maintain 90% viability during the probiotic

harvesting procedure.

Furthermore, when combined with other ingredients to create probiotic products, these spores maintain their stability at a concentration of  $1 \times 10^9$  spores/mL, with a possible five-year shelf life. Moreover, spores continue to be viable during the production of poultry feed (Ramlucken et al., 2020; Ogbuewu et al., 2022). This enables the production of probiotic-enriched diets, which are likewise made using granulation (Krysiak et al., 2021). The probiotic family is entering a new chapter with the identification of many fungal strains that function as probiotics. Fungi are important probiotic possibilities due to their distinct cellular architecture and superior capacity to survive in the harsh environment of the gastrointestinal tract. The use of novel fungal strains in therapy is not entirely proven since the precise mode of action, level of efficacy, and dose are still unknown. *Saccharomyces boulardii* var. *cerevisiae* is the most promising marketable probiotic yeast strain among the fungal strains isolated as potential probiotic candidates; it has multiple health benefits in both favorable and unfavorable physiological states of the host body (Banik et al., 2019).

### **Probiotics in poultry**

Even though probiotics are frequently used in animal husbandry, the major problem with their application is that some probiotics have antibiotic-resistance genes, especially those encoded by plasmids that can be transferred between organisms. Therefore, probiotic companies should perform an antibiotic susceptibility test for multiple antibiotics before considering a microorganism as a probiotic candidate. In addition, they should perform an analysis of the complete genome sequence for prediction of antibiotic resistance genes (Fatahi-Bafghi et al., 2022). Furthermore, label accuracy related to microorganism identification and concentration in these commercial products, as total viable cells, is a serious issue that can have a significant impact on the effectiveness of the product and the health of chickens. Furthermore, most studies use a comparison of mono-treated animals with non-treated controls to assess the efficacy of probiotics. The impact that different vaccines, feed, and other factors may have on an animal's response to the live prophylactic of interest is not considered, even though this experimental design is an essential first step in

determining the usefulness of a live prophylactic.

Further research is also necessary to determine whether these live prophylactics can influence animal behavior through the gut-brain axis, offering a practical way to enhance social behaviors in poultry flocks (Redweik et al., 2020a, b). Due to the low resistance of microorganisms to gastrointestinal digestion, many probiotic products containing carefully chosen bacteria may not work as intended. One of the most promising methods for shielding probiotics from unfavorable environmental circumstances appears to be microencapsulation (Babot et al., 2023). Probiotics are mostly given on chicken farms by adding them to feed, but there are numerous alternative ways as well, including sprays, granules, tablets, coated capsules, gavages (vaccines or drops), and powder sachets. Growers are choosing to add formulations to the water in addition to adding probiotics to the feed (Krysiak et al., 2021). Commercial probiotics available for poultry application can be divided into three classes: bacteria, yeasts, and a mixture of bacteria and yeast. Each class can be composed of one or more strains belonging to the same class (Table 1). We will detail some of them in this review.

The probiotic supplement FloraMax<sup>®</sup>-B11 is made up of *Pediococcus parvulus* and *Ligilactobacillus (ex-Lactobacillus) salivarius*. Broilers fed FloraMax<sup>®</sup>-B11 exhibited decreased colonization of *Salmonella* serovar Enteritidis upon oral challenge, enhanced gut barrier function, and decreased peripheral blood percentages of heterophils, lymphocytes, eosinophils, and basophils in comparison to control broilers (Prado-Rebolledo et al., 2017). Considering the significance of immune inflammation in eliminating intestinal *Salmonella* spp. and the noted decrease in circulating immune cells, it is plausible that this product has directly decreased the amount of *Salmonella* spp present in the gut (Kogut et al., 1994). Each probiotic bacterium in FloraMax<sup>®</sup>-B11 directly decreased the growth of *Salmonella* ser. Enteritidis, *E. coli*, and *Campylobacter jejuni* *in vitro*, supporting the mechanism of direct competition (Menconi et al., 2014). Furthermore, this product decreased intestinal gene expression linked to the NF $\kappa$ B complex and aldose reductase (Higgins et al., 2011), indicating that this probiotic also lowers the

expression of genes related to inflammation. FloraMax<sup>®</sup>-B11 enhanced gut morphology and greatly reduced *Salmonella* spp recovery, incidence, and horizontal transmission to broiler chicks when combined with the perinatal supplement EarlyBird (Pacific Vet Group USA Inc.) (Biloni et al., 2013). *In ovo*, administration of FloraMax<sup>®</sup>-B11 did not negatively impact the ability of herpesvirus of turkeys (HVT) vaccine to protect against Marek's disease or hatchability of chickens, but improves body weight during the first seven days of life and decreases *Salmonella* ser. Enteritidis recovery in chickens (Teague et al., 2017). Finally, after being challenged with *Clostridium perfringens*, the causative agent of necrotic enteritis, broilers supplemented with FloraMax<sup>®</sup>-B11 demonstrated notable increases in body weight, decreased levels of total *C. perfringens*, and decreased necrotic enteritis-induced mortality (Layton et al., 2013) and it did not negatively impact bile acid metabolism and enterohepatic circulation, which appeared to be age-dependent (Kpodo et al., 2022).

One LAB, *Enterococcus faecium* NCIMB 10415 (Mirza, 2018), makes up Cylactin<sup>®</sup>. When broilers were fed Cylactin<sup>®</sup>, their average body weight increased, the amount of *E. coli* and *Clostridium* spp. in feces and intestinal tract significantly decreased when compared to controls, and their production of lactate and short- and branched-chain fatty acids was enhanced (Slizewska et al., 2020). Nevertheless, the intestinal mucosa *Salmonella* ser. Enteritidis load was not decreased by Cylactin<sup>®</sup> (Beirão et al., 2018). Despite being examined in a non-avian setting, Cylactin<sup>®</sup> supplementation in the piglets' food resulted in a notable decrease in mucus-adherent extraintestinal pathogenic strains of *E. coli* (Bednorz et al., 2013), indicating that this probiotic may directly impact avian pathogenic *E. coli* (APEC) present in the chicken gut. *Enterococcus faecium* NCIMB 10415 is free of known virulence factors. Resistance to kanamycin shown in this strain is most likely to be caused by an unknown mechanism that potentiates the effect of the *sfkmr* gene and not by the acquisition of genes coding for aminoglycosides-modifying enzymes and is thus not a cause for concern. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) considers that the additive is safe for the chickens at the proposed conditions of use.

**Table 1:** Summary of commercial probiotics available for poultry application based on (Markowiak and Śliżewska, 2018; Redweik et al., 2020a, b). It includes the new names for some ex-*Lactobacillus* genera (Zheng et al., 2020).

Probiotic class	Trade name of the preparation (producer)	Number of strains	Microorganisms (per label)
Bacteria	Cylactin® (DSM)	1	<i>Enterococcus faecium</i> (NCIMB 10415)
	Fecinor®, Fecinor® soluble and Fecinor® soluble plus (Evonik)	1	<i>Enterococcus faecium</i> (CECT 4515)
	B.I.O.Sol (Biochem)	1	<i>Enterococcus faecium</i>
	Galvit Probiotyky (Galvit)	1	<i>Enterococcus faecium</i>
	Lactifer® (Chr Hansen)	1	<i>Enterococcus faecium</i>
	Oralin® (Chevita GmbH)	1	<i>Enterococcus faecium</i>
	Propoul (International Probiotic Company)	1	<i>Limosilactobacillus fermentum</i>
	Probiomix B	2	<i>Lactiplantibacillus plantarum</i> (KKP/593/p), <i>Lactocaseibacillus rhamnosus</i> (KKP 825) <sup>1</sup>
	Acid-Pak-4-Way (Alltech)	2	<i>Lactobacillus acidophilus</i> , <i>Enterococcus faecium</i>
	Farmafore soluble (Farm'apro)	2	<i>Lactocaseibacillus rhamnosus</i> , <i>Companilactobacillus farciminis</i>
	Avian PAC (Soluble Loveland Industries)	2	<i>Streptococcus faecium</i> , <i>Lactobacillus acidophilus</i>
	FloraMax®-B11 (Pacifc Vet Group)	2	<i>Pediococcus parvulus</i> , <i>Ligilactobacillus salivarius</i>
	Cerbiogalli	3	<i>Lactobacillus acidophilus</i> , <i>Lactocaseibacillus casei</i> , <i>Lactiplantibacillus plantarum</i>
	Biomix-PoultryStar® (DSM)	4	<i>Enterococcus faecium</i> , <i>Limosilactobacillus reuteri</i> , <i>Pediococcus acidilactici</i> , and <i>Bifidobacterium animalis</i>
	GalliPro® (DSM)	1	<i>Bacillus subtilis</i> (DSM 17229)
	Calsporin (ORFFA)	1	<i>Bacillus subtilis</i>
	Enviva™ Pro (DANISCO Animal Nutrition)	1	<i>Bacillus subtilis</i>
	CloSTAT® (Kemin)	1	<i>Bacillus subtilis</i> (PB6)
	EcobioI® (Evonic)	1	<i>Bacillus amyloliquefaciens</i> (CECT 5940)
	Bio Plus2B® (Chr. Hansen)	2	<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i>
	GalliPro® Fit (DSM)	3	<i>Bacillus subtilis</i> (DSM 32324 and DSM 32325), <i>Bacillus amyloliquefaciens</i> (DSM 25840)
	Norum™ (Eco-Bio/Euxxis Bioscience LLC)	3	<i>Bacillus amyloliquefaciens</i> (AM0938 and JD17), <i>Bacillus subtilis</i>
	Zymospore® (Vetanco)	3	<i>Bacillus subtilis</i> (BS009, BS020, BS-024)
	Yeast	Levucell® SB (Lalleman Animal Nutrition)	1
Mixture of bacteria classes or/and yeast	Biogen D (Bio-Gen)	3	<i>Bifidobacterium bifidum</i> , <i>Lactobacillus acidophilus</i> , <i>Pediococcus faecium</i>
	Probiomix (Woogene B&G Co. Ltd.)	3	<i>Bacillus subtilis</i> , <i>Clostridium butyricum</i> , <i>Lactobacillus acidophilus</i>
	Doctor Em® (Biotron)	4	<i>Lactocaseibacillus paracasei</i> , <i>Lactiplantibacillus plantarum</i> , <i>Lactococcus lactis</i> , <i>Saccharomyces cerevisiae</i>
	PrimaLac® (Star Labs, Inc.)	4	<i>Lactobacillus acidophilus</i> , <i>Lactocaseibacillus casei</i> , <i>Enterococcus faecium</i> , <i>Bifidobacterium bifidum</i>
	Lavipan® (JHJ)	5	<i>Lactococcus lactis</i> (IBB 500), <i>Carnobacterium divergens</i> (S-1), <i>Lactocaseibacillus casei</i> (LOCK 0915), <i>Lactiplantibacillus plantarum</i> (LOCK 0862), <i>Saccharomyces cerevisiae</i> (LOCK 0141)
	Gro-2-Max® (BioNatural America Institute)	5	<i>Lactobacillus acidophilus</i> , <i>Pediococcus pentosaceus</i> , <i>Pediococcus acidilactici</i> , <i>Bacillus subtilis</i> , <i>Saccharomyces cerevisiae</i>
	Probiomix (Chr Hansen)	6	<i>Lactobacillus acidophilus</i> , <i>Lactocaseibacillus casei</i> , <i>Lactiplantibacillus plantarum</i> , <i>delbrueckii subsp. lactis</i> , <i>Enterococcus faecium</i> , <i>Bacillus subtilis</i>
	MicroGuard® (PeterLab Holdings)	11	<i>Bacillus licheniformis</i> , <i>Bacillus megaterium</i> , <i>Bacillus mesentericus</i> , <i>Bacillus polymyxa</i> , <i>Bacillus subtilis</i> , <i>Bifidobacterium bifidum</i> , <i>Lactobacillus acidophilus</i> , <i>Lactobacillus delbrueckii subsp. bulgaricus</i> , <i>Lactiplantibacillus plantarum</i> , <i>Streptococcus faecium</i> , <i>Saccharomyces boulardii</i>
	Pro-Biotyky em15® (ProBiotics)	12	<i>Bacillus subtilis</i> , <i>Bifidobacterium animalis</i> , <i>Bifidobacterium bifidum</i> , <i>Bifidobacterium longum</i> , <i>Lactobacillus acidophilus</i> , <i>Lactocaseibacillus casei</i> , <i>Lactobacillus delbrueckii subsp. bulgaricus</i> , <i>Limosilactobacillus fermentum</i> , <i>Lactiplantibacillus plantarum</i> , <i>Lactococcus lactis subsp. lactis</i> , <i>Saccharomyces cerevisiae</i> , <i>Streptococcus thermophilus</i>

<sup>1</sup> EFSA FEEDAP Panel (2016).

This conclusion is extended to chickens reared for laying and extrapolated to minor poultry species for fattening and minor poultry species reared for laying. *E. faecium*. Cylactin® is not a skin/mucosal irritant or skin sensitizer, and it is compatible with decoquinatate, monensin, robenidine, diclazuril, semduramycin, lasalocid A sodium, maduramycin ammonium, narasin, narasin/nicarbazin and salinomycin. As the product is formulated with large particle sizes and the dusting potential is low, exposure of users will be minimal (EFSA Panel FEEDAP, 2010; 2014).

Fecinor® is a preparation of a viable strain of *Enterococcus faecium* CECT 4515 currently authorized in the EU for use in feeds for chickens (and weaned piglets) for fattening at the level of  $1 \times 10^9$  colony-forming units (CFU)/kg. *E. faecium* CECT 4515 is free of known enterococcal virulence factors and does not harbor acquired genes coding for antibiotic resistance. Furthermore, this bacterium is not a skin or eye irritant and does not cause skin sensitization and it has the potential to improve body weight gain and feed-to-gain ratio when used at the recommended dose. The use of this product is permitted in feed containing one of the authorized coccidiostats: monensin sodium, diclazuril, nicarbazin, decoquinatate, robenidine hydrochloride, semduramycin sodium, narasin, salinomycin sodium, lasalocid sodium narasin/nicarbazin or maduramycin ammonium (Barroso, 2011; EFSA FEEDAP Panel, 2011). The application was approved for Fecinor® soluble and Fecinor® soluble plus, two new formulations that differ by the replacement of one excipient, for use in drinking for chickens for fattening at  $5 \times 10^8$  CFU/L. Fecinor®, Fecinor® soluble, and Fecinor® soluble plus are considered safe for consumers, users, and the environment (EFSA FEEDAP Panel, 2015).

The strain of *Bacillus subtilis* DSM 17229, which makes up GalliPro®, has been shown to enhance performance and decrease ammonia emission from the excreta in broilers (Upadhaya, 2019). This product can reverse the decrease of splenic mass in chickens infected with *Salmonella* spp.; however, feeding this probiotic to non-infected birds did not alter any immunological markers (Sadeghi et al., 2015). Additionally, GalliPro® improved the utilization of crude protein from the diet, which reduced the expense of feeding broilers and boosted body

weight and feed conversion ratios (Zaghari et al., 2015). However, this study did not demonstrate whether GalliPro® contributed to this liberation directly or indirectly through altering the microbiota. In comparison to control broilers, the addition of GalliPro® to feed significantly decreased the amount of *Salmonella* spp. in drag swabs and cecum samples (Knap et al., 2011).

Furthermore, broilers that received GalliPro® in feed diet gained more weight, converted feed more efficiently than control, and eliminated *C. perfringens* from the ileum but not from the caecum (Abudabos et al., 2015). On the other hand, GalliPro® Fit (*Bacillus subtilis* DSM 32324, *B. subtilis* DSM 32325 and *Bacillus amyloliquefaciens* DSM 25840), used as a zootechnical additive in feed and water for all poultry species for fattening or reared for laying/breeding, has two bacterial species that was presumed safe for the target species, consumers, and the environment by EFSA FEEDAP Panel. The identity of the active agents was established, and the lack of toxigenic potential was confirmed. The strains did not show resistance to relevant antibiotics. The Panel concluded that GalliPro® Fit is compatible with diclazuril, decoquinatate, and halofuginone, and it has the potential to be efficacious in chickens for fattening at  $1.6 \times 10^9$  CFU/kg feed and at  $5.4 \times 10^8$  CFU/L water for drinking. This conclusion was extrapolated to all other poultry species for fattening or rearing for laying/breeding (EFSA FEEDAP Panel et al., 2020).

CloSTAT® is a feed additive composed of a strain of *B. subtilis* PB6. In comparison to *C. perfringens*-challenged broilers without probiotics, challenged animals consuming CloSTAT® at  $1 \times 10^9$  CFU/g of feed had significantly higher body weight and feed intake. Nevertheless, neither the lactobacilli nor the *C. perfringens* bacterial burden in the ileal digesta was considerably altered by CloSTAT® supplementation (Khalique, 2017). Furthermore, feed supplementation with this probiotic at  $4 \times 10^7$  CFU/kg reduced the lesion score of challenged chicks, with increased tight junction-related gene expression (occludin and ZO-1) and decreased tumor necrosis factor (TNF- $\alpha$ ) expression compared with *C. perfringens* -infected birds. A decrease in the abundance of *Clostridium* XI, *Streptococcus*, and *Staphylococcus* was observed after *C. perfringens* infection, while supplementation with CloSTAT® restored the ileal

microbial composition (Liu et al., 2021). When broilers fed CloSTAT<sup>®</sup>, control, and antibiotic growth promoters were examined for their *E. coli* challenge mortality rates, CloSTAT<sup>®</sup> demonstrated a reduction like that of the antibiotic growth promoter (both significantly compared to control) (Teo et al., 2006). CloSTAT<sup>®</sup>, like GalliPro<sup>®</sup>, decreased the colonization of the ileum by *C. perfringens* after a challenge (Abudabos et al., 2013).

The product called Norum<sup>™</sup> (Eco-Bio/Euxxii Bioscience LLC, Fayetteville, AR), a *Bacillus* spore DFM culture, consists in 3 isolates: 2 strain of *Bacillus amyloliquefaciens* (AM0938 and JD17) and one strain of *Bacillus subtilis* (Latorre et al., 2016). The addition of Norum<sup>™</sup> in poultry diets improved body weight, body weight gain, and feed conversion (Hernandez-Patlan et al., 2019; Solis-Cruz et al., 2019). This product reduced necrotic enteritis lesion scores, *C. perfringens* load, IgA levels, fluorescein isothiocyanate-dextran serum levels, phylum *Proteobacteria*, and the genus *Clostridium*, *Turicibacter*, *Enterococcus*, and *Streptococcus*, whereas *Lactobacillus* and *Bacillus* were increased in the Norum<sup>™</sup> group as compared to control. Finally, adding Norum<sup>™</sup> *in ovo* reduced the risk of virulent *E. coli* spreading horizontally and infecting broiler chicks during hatching significantly, possibly by changing the composition and community structure of the microbiota (Arreguin-Nava et al., 2019). Research on broilers and turkeys given a rye-based diet revealed that the addition of Norum<sup>™</sup> enhanced growth performance, bone mineralization, and microbiota composition, as well as reduced digesta viscosity and bacterial translocation. When intestinal viscosity was lowered by adding this product, intestinal inflammation, and bacterial translocation were reduced as well, suggesting that the supplemented groups absorbed more nutrients through the intestinal brush border (Latorre et al., 2014, 2015; Tellez et al., 2020). These differences could be caused by fewer substrates available for bacterial growth. The notable performance improvements could be explained by the production of enzymes from the combined strains of *Bacillus* spp used in this product that can increase nutrient absorption, promoting growth performance and more efficient feed conversion (Hernandez-Patlan et al., 2022). Also, this product reduced the ammonia

concentration in turkey manure (Tellez et al., 2020). Furthermore, Research has demonstrated that this DFM considerably lessens the severity of aflatoxicosis (Solis-Cruz et al., 2019) and *Salmonella* ser. Enteritidis experimental infections (Adhikari et al., 2019).

Zymospore<sup>®</sup> (Vetanco, Villa Martelli, Argentina), a probiotic composed of 3 *Bacillus subtilis* strains, was tested in two *in vivo* experiments with broilers. In order to cause intestinal dysbiosis, broilers were administered a specific challenge containing *Eimeria* spp. and *Clostridium perfringens*. The ideal dose of this probiotic was 0.3 kg/ton, where broilers performed as well as Flavomycin<sup>®</sup> treatment. Additionally, the study of the intestinal microbiome shows that using this probiotic improved feed digestion and encouraged growth performance while increasing bacterial diversity. In another experiment, broilers were grown on recycled litter and given an oral challenge to replicate commercial growth settings. The probiotic and the birds fed 11% bacitracin methylene disalicylate both performed well in the study (de Souza et al., 2022).

The ingredients of PrimaLac<sup>®</sup> include  $1 \times 10^6$  CFU/g of *Lactobacillus acidophilus*, *Lactocaseibacillus (ex-Lactobacillus) casei*, *Enterococcus faecium*, and *Bifidobacterium bifidum*. It has been demonstrated that using PrimaLac improved poultry performance, significantly increased bacteria *Lactobacilli* in cecal content, and reduced the colonization of *Campylobacter jejuni*, *Clostridium perfringens*, and *Salmonella* spp. (3 *Salmonella* serotypes: Typhimurium, Kentucky, and Heidelberg) in broiler chickens, and increased immune system efficiency (Abudabos, 2012; Embrahami et al., 2015, 2016; Grimes et al., 2008). Also, this probiotic increased goblet cell (GC) numbers, total GC area, GC mean size, mucosal thickness, and the number of segmented filamentous bacteria compared with controls in turkeys (Rahimi et al., 2009). When this probiotic was given to broilers *in ovo*, it did not impact hatchability, but it improved performance during the first-week post-hatch, and it was capable of modulating gene expression in the ileum and cecal tonsils (Pender et al., 2017). Furthermore, although administration of this probiotic appeared to improve the antibody responses to Newcastle disease virus and infectious bursal disease vaccination in chickens, the antibody

titers of the probiotic-treated group were not significantly different from those not receiving probiotics (Talebi et al., 2008).

Levucell® SB is a feed additive consisting of viable cells of a strain of *Saccharomyces cerevisiae* (CNCM I-1079) currently authorized by the European Food Safety Authority (EFSA) as a zootechnical additive for chicken, turkey, and minor poultry species as well as for piglets, and sows (Commission Regulation, 2018; EFSA FEEDAP Panel et al., 2017, 2019). The EFSA concluded that there is some evidence that the addition of Levucell® SB to poultry diets has the potential to aid the reduction of carcass contamination with *Salmonella* spp. and so improve the quality of poultry products. The effective dose appears to be  $2 \times 10^{10}$  CFU/kg of feed. This conclusion can be extrapolated to minor avian species for fattening when used at the same dose but not to minor poultry species for laying (EFSA FEEDAP Panel et al., 2017).

The probiotic Lavipan® includes LAB and one strain of yeast: *Lactococcus lactis* IBB500 (origin - chicken feces), *Carnobacterium divergens* S-1 (origin - carp gut), *Lacticaseibacillus (ex-Lactobacillus) casei* LOCK 0915 (origin - chicken feces) and *Lactiplantibacillus (ex-Lactobacillus) plantarum* LOCK 0862 (origin - turkey feces) in the amount of  $1 \times 10^9$  CFU/g each and *Saccharomyces cerevisiae* LOCK 0141 (origin - plant silage) in the amount of  $1 \times 10^7$  CFU/g. Lavipan® added to a feed for broiler chickens, was capable of reducing the extent of *Campylobacter* spp. and *Salmonella* ser. Enteritidis is an invasion in the gastrointestinal tract of birds, and it has encouraged immunomodulatory characteristics that could successfully increase the efficacy of the particular prophylactic regimen used in a flock of broiler chickens (Smialek et al., 2018, 2019). In comparison to the control group, this probiotic also enhanced the villus morphometric characteristics (i.e., villus width and surface area) in the ileum, jejunum, and duodenum (Bogucka et al., 2019). EFSA reported that the identity of all strains had been established and no antibiotic resistance of concern detected, and these strains are presumed safe for poultry (and weaned piglets), consumers of products from animals fed the additive, and the environment. Lavipan® should be taken into consideration as a possible respiratory sensitizer, even if it is not harmful by inhalation or an irritant to the skin

or eyes. Furthermore, this probiotic has the potential to improve the performance of chickens for fattening when supplemented at the recommended dose of  $5 \times 10^8$  CFU LAB/kg of feed and  $5 \times 10^6$  CFU *S. cerevisiae*/kg of feed, and it is compatible with diclazuril, salinomycin sodium, decoquinat, maduramicin and narasin+ nicarbazin (EFSA FEEDAP Panel, 2016).

MicroGuard® includes 11 microorganisms (Markowiak and Śliżewska, 2018), ten bacteria (*Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mesentericus*, *Bacillus polymyxa*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactiplantibacillus-ex-Lactobacillus-plantarum*, and *Streptococcus faecium*) and one yeast (*Saccharomyces boulardii*). Increases in final body weight, weight gain, high-density lipoprotein, triglyceride, and antibody titers against Newcastle disease (ND) and avian influenza (AI) were observed in chickens given MicroGuard® at 150 g/ton. Probiotic-supplemented broilers showed reduced plasma levels of gamma-glutamyl transpeptidase, alkaline phosphatase, and alanine aminotransferase, as well as improved feed conversion ratio, increased villus height, and villus height to crypt depth ratio. However, groups fed 100 or 150 g/ton of this probiotic decreased carcass yields, liver weights, breast muscle values, and abdominal fat weights. *Salmonella* spp, *Escherichia coli*, and ileal coliform counts dropped in groups fed 100 or 150 g/ton of MicroGuard® (Manafi et al., 2018). Furthermore, this probiotic improved different haematobiochemical parameters in broiler chickens (Rahman et al., 2013).

Gro-2-Max® (BioNatural America Institute) is a multi-species supplement of LAB (*Lactobacillus acidophilus*, *Pediococcus pentosaceus*, *P. acidilactici*), *Bacillus subtilis*, and *Saccharomyces cerevisiae*. Gro-2-Max® supplementation by feed or water had a positive effect on chicken performance, decreasing the effect on lipogram, especially total cholesterol, total triglycerides, and low-density lipoprotein cholesterol, nonspecific humoral and cellular immune responses, and improving the effect on intestinal function through increasing the height of ileal villi (Abd El-Baky et al., 2016). Using this probiotic alone versus in combination with a recombinant attenuated *Salmonella* vaccine (RASV; VAX) has major implications for catecholamine production

and the microbiota of layer pullets (Redweik et al., 2020a). Additionally, when administered both recombinant attenuated *Salmonella* vaccine and Gro-2-Max<sup>®</sup>, layers showed improved resistance to both avian pathogenic *Escherichia coli* and *Salmonella* serotype Kentucky, indicating that this probiotic has adjuvant properties (Redweik et al., 2020b). The effectiveness of Gro-2-Max<sup>®</sup> on ESBL-producing *E. coli* (O78) isolate transmission and excretion was studied *in vivo*, and this product diminished the cecal colonization of extended-spectrum  $\beta$ -lactamase *E. coli* isolates in broilers with significant minimization and downregulation for involved genes in biofilm synthesis (Ebrahim et al., 2023).

### Prebiotics

Gibson and Roberfroid introduced the concept of “prebiotics” in 1995 (Gibson and Roberfroid, 1995). In the decades that followed, research on prebiotics tended to center on finding substrates that specifically target gut bacteria that are known to promote health, such as lactobacilli and bifidobacteria. In December 2016, a panel of experts in microbiology, nutrition, and clinical research was convened by the ISAPP to review the definition and scope of prebiotics. They updated the definition of a prebiotic, which is currently in agreement: “a substrate that is selectively utilized by host microorganisms conferring a health benefit”. The idea thus consists of three fundamental components: a substance, a physiologically beneficial effect, and a microbiota-mediated mechanism (Gibson et al., 2017).

Although prebiotics and dietary fibers are sometimes confused, only a portion of dietary fibers meet the criteria for being prebiotics, which can also originate from non-fiber materials like polyphenols. A prebiotic substance must, by definition, have a positive physiological effect on the host, and that benefit must result, at least in part, from the compound's consumption by local bacteria. Prebiotics were initially targeted at resident lactobacilli and bifidobacteria, but studies on the microbiome have revealed other types of health-related microbes that could also be prebiotic targets. But in order to fit the requirements of this definition, the prebiotic material must only have an impact on a specific subset of the host's microbes, not the entire microbial ecology

(Gibson et al., 2017).

Prebiotics are not yet a term recognized by the Food and Drug Administration in the USA, and they are regulated based on the category of product their intent and design dictates (Gibson et al., 2017). On the other hand, the Global Prebiotic Association is comprised of ingredient manufacturers, brand holders, and retailers focused on raising awareness of and support for the prebiotic category. It has the mission of “Increase public awareness about the production, quality, and science of prebiotic products, expand manufacturer understanding of the solid science supporting both well-known and newfound benefits, and create needed transparency about product quality” (Global Prebiotic Association, 2023).

### Sources and mechanism of action of prebiotics

Prebiotics can exist in synthetic or natural forms. They have received less attention in most studies on their health effects as components of whole plant foods, with the focus being on isolated compounds (allowing for more stringent control over substance and dose). Prebiotics have been investigated for their potential to improve immunological function, cardiometabolic health, infection prevention, and mineral availability. Currently established prebiotics are carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids might fit the updated definition, assuming convincing weight of evidence in the target host (Gibson et al., 2017).

There are five categories of prebiotics: fructans, galacto-oligosaccharides (GOS), starch and glucose-derived oligosaccharides, other oligosaccharides, and non-carbohydrate or miscellaneous (Table 2). Fructans consist of inulin and fructo-oligosaccharide (FOS) or oligofructose. GOS, the product of lactose extension, are classified into two subgroups: (i) the GOS with excess galactose at C3, C4, or C6 and (ii) the GOS manufactured from lactose through enzymatic trans-glycosylation. There are some GOSs derived from lactulose, the isomer of lactose. There is a kind of starch that is resistant to upper gut digestion known as resistant starch (RS). Polydextrose is a glucose-derived oligosaccharide. It consists of glucan with a lot of branches and glycosidic linkages. Among other oligosaccharides, some of them are called pectic

oligosaccharides. They are based on the extension of galacturonic acid (homogalacturonan) or rhamnose (rhamnogalacturonan I). Among the non-carbohydrate category, cocoa-derived flavanols, polyphenolics, fatty acids, herbs, and other supplements (micronutrients) are some of them (Ananda et al., 2022; Davani-Davari et al., 2019; Bamigbade et al., 2022).

The most often used prebiotics in poultry

include FOS, mannan-oligosaccharides (MOS), and GOS. Fructose, which is present in a variety of plants, including onion, chicory, garlic, asparagus, bananas, and artichokes, among others, is converted into FOS. GOS polymers can be produced by  $\beta$ -galactosidase's enzymatic hydrolysis of lactose. In addition to producing fermentation products with potential prebiotic qualities, the yeast *Saccharomyces cerevisiae* is a source of MOS (Ricke et al., 2020).

**Table 2:** Categories of prebiotics (Davani-Davari et al., 2019; Bamigbade et al., 2022).

Types of prebiotics	Subgroups	Examples	Functions
Fructans	-	Inulin and fructooligosaccharides or oligofructose	Selective stimulation of lactic acid bacteria
Galactooligosaccharides (GOS)	GOS with excess galactose at C <sub>3</sub> , C <sub>4</sub> or C <sub>6</sub>  GOS manufactured from lactose through enzymatic trans-glycosylation	GOS	Stimulation of <i>Bifidobacteria</i> and <i>Lactobacilli</i>
Starch and glucose-derived oligosaccharides	-	Resistant starch, polydextrose, mannan-oligosaccharides	Butyrate production, stimulation of <i>Bifidobacteria</i>
Other oligosaccharides	-	Pectic oligosaccharide	
Non-carbohydrate or miscellaneous	-	Cocoa-derived flavanols, polyphenolics, fatty acids, herbs, and other supplements	Modulation of microbial diversity, cell membrane integrity

Although fermentable carbohydrates in the gut are the prebiotic substances with the most well-studied health effects to date, a wide variety of chemicals targeting other host niches (e.g., the mouth, skin, or urogenital tract) can be classified as prebiotics according to the mainstream definition. Furthermore, the beneficial effect(s) of a prebiotic on health must be confirmed in the target animal for its intended use and mediated through the microbiota (Gibson et al., 2017). There are two main ways in which prebiotics may work once they are incorporated into the host. The matching prebiotic first enters the chicken's gut without breaking down in the upper gastrointestinal system, but bacteria that are thought to be advantageous to the host use them specifically. Second, the prebiotic's presence contributes to additional gut activities such as the production of lactic acid and short-chain fatty acids as byproducts of microbial fermentation, a slower rate of pathogen colonization, and possibly even health benefits for birds (Ricke et al., 2020).

### Prebiotics in poultry

Several commercial prebiotics have been studied and utilized, such as Biolex<sup>®</sup> MB40, Leiber<sup>®</sup> ExCel (Leiber, Hafenstrasse 24, Germany), which are brewer's yeast cell walls composed of MOS, Bio-MOS<sup>®</sup>, Fasttrack<sup>®</sup> (Fasttrack, Conklin, Kansas City MO) and PoultryStar<sup>®</sup> (PoultryStar, BIOMIN GmbH, Herzogenburg, Austria), contain FOS, Bimuno (Clasado Ltd), contain GOS, and Fermacto, which is a meal of *Aspergillus oryzae* (PetAg, USA) (Micciche et al., 2018; Zahirian et al., 2019).

The effect of prebiotics added to poultry diets is probably dependent on the specific prebiotics' chemical makeup and the metabolic capacities of the microbiota living in the chicken gut. Prebiotics are thought to be hydrolyzed and then used by the GIT bacteria found in different parts of the avian GIT because they have been described as indigestible by the host. Dietary fibers and other undigested food items typically pass through the upper sections of the GIT and

into the ceca, where they serve as a source of nutrition for the local microbiota (Svihus et al., 2013). Several attributes have been associated with prebiotic supplementation in conventional poultry production. One of the more characterized benefits is the development of GIT microorganisms that decrease the likelihood of early colonization of pathogens in young birds. In general, young birds are more vulnerable to enteric pathogens due to their lack of GIT microbiota that can compete with incoming pathogens such as *Salmonella* spp. The support of beneficial bacteria in the chicken GIT elicits preventive mechanisms against foodborne pathogens through microbial metabolism and results in altered immune responses of the host due to the GIT microbial population changes (Ricke, 2021).

Prebiotics provide a dietary way to choose GIT bacteria that may operate as a barrier against the colonization of foodborne pathogens like *Salmonella* spp. and *Campylobacter*, in addition to improving the GIT host health (Kim et al., 2019; Micciche et al., 2018). The capacity of prebiotics to boost the amount of LAB in the stomach may facilitate pathogens' competitive exclusion from birds' gastrointestinal tracts (Pourabedin et al., 2015). Prebiotics increase intestinal acidity, which may also aid in lowering hens' intestinal illnesses and strengthen hens' immune responses to speed up the removal of infections (Ajuwon, 2016). Furthermore, they have the potential to influence immune cells directly or indirectly in the gut by favoring the colonization of advantageous bacteria and microbial metabolites (Collins and Gibson, 1999; Pandey et al., 2015; Teng and Kim, 2018).

Administering prebiotics increased the relative abundance of beneficial bacteria and eradicated pathogenic ones in the birds' gut microbiome even in heat stress conditions (at weeks 4 and 5, temperature sets at 32–35°C, Sayed et al., 2023). It has been shown that prebiotics in broiler feed increase the number of lactobacilli. Bifidobacteria was shown to be more prevalent and clostridia to be less prevalent in certain studies on the microbiological effects of prebiotic supplementation (Van den Broek et al., 2008; Shehata et al., 2022), and there was a slight decrease in coliforms and *Salmonella* spp. (Dhama et al., 2008; Janssens et al., 2004). Intestinal morphology in broiler diets indicated that prebiotics raised the height of the intestinal

villus. In the digestive system, a healthy population of these beneficial bacteria augments the processes of detoxification and excretion (Teitelbaum et al., 2002). Also, prebiotics have been shown to enhance the quality of eggshells and bones, enhance the consumption of minerals, and enhance the performance of laying hens (Yalçin et al., 2014a).

Chitosan oligosaccharides (COS) is a relatively new feed additive that is a derivative of chitosan, a non-toxic linear polysaccharide with many biological functions. Low to medium doses are preferable for safe poultry production because they improve growth performance, increase villus surface area, decrease undesirable cholesterol, and positively affect blood glucose and protein levels (Ayman et al., 2022). COS may also be helpful in lessening the detrimental effects of stress on the gut health of broiler chickens (Osho and Adeola, 2020).

From the fermentation of *Aspergillus oryzae*, it is possible to obtain *Aspergillus* meal (AM). AM can be utilized to improve performance in commercial poultry diets with low protein levels because it contains 16% protein and 44% fiber (Harms et al., 1988; Torres-Rodriguez et al., 2005). Beta-glucans, FOS, chitosan, and MOS are also present in AM. This prebiotic source helps chickens grow, too, perhaps by improving the digestion and absorption of feed ingredients (Hernandez-Patlan et al., 2018). It has been shown that feeding AM to turkey poults changes their intestinal morphometry. When compared to the control, it enhanced the villi height and surface area in the duodenum and ileum of turkey poults as well as the amount of acid mucin cells, neutral mucin cells, and sulphomucin cells (Tellez et al., 2010). Other studies indicated that giving neonate poults AM prebiotic for 30 and 42 days increased body weight, carcass composition, and health and improved feed conversion, but these positive effects were mainly reached by adding AM for the entire rearing period (Amirdahri et al., 2012; Zahirian et al., 2019). Furthermore, chicks fed dietary AM prebiotics exhibited reduced ileum energy and protein content in comparison to control chicks, suggesting enhanced food absorption and digestion (Reginatto et al., 2011). Feeding broiler chickens and turkeys with 0.2% AM decreased overall colonization levels, which in turn decreased horizontal *Salmonella* Typhimurium and *S. Enteritidis* transmission (Londero et al.,

2011). One possible explanation for the decrease in *Salmonella* spp. colonization could be the combined action of beta-glucan, MOS, chitosan, and FOS present in the mycelium of *Aspergillus oryzae*.

MOS supported live performance equivalent to bacitracin methylene disalicylate followed by virginiamycin and had an additive effect when combined with the antibiotics (Hooge et al., 2003). It decreases the load of pathogenic bacteria through i) binding bacterial type-1 fimbriae, ii) increasing goblet cells, which produce bactericidal mucin, and 3) providing a favorable environment for the growth of beneficial bacteria leading to competitive exclusion (Chacher et al., 2017). FOS supplementation did not have any detrimental effects on molting performance (Kim et al., 2006) but increased bone mineral concentrations as well as intestinal absorption of calcium and magnesium (Scholz-Ahrens et al., 2007).

Yalçın et al. (2014b) observed that the reduction of abdominal fat, improvement in humoral immune response, and enhanced growth performance of broilers were the results of the dietary inclusion of yeast cell walls generated from baker's yeast. Furthermore, this yeast cell wall improved humoral immune response and produced low-cholesterol eggs in another trial using laying hens (Yalçın et al., 2014a).

A management technique that needs to be decided upon is when to add prebiotics to the diet of poultry. Considering that diseases like that produced by *Salmonella* spp. can develop in very young chicks, it might make sense to give prebiotics to birds at a reasonable age. Prebiotics have generated some interest, and this could be a useful tactic for promoting the early formation of a resident GIT microbial community that is more pathogen-hostile. This could depend on the kind of prebiotic being provided; thus, it would be necessary to figure out the right amounts. How this might affect the growth of the chicks and the amount of time the prebiotic is exposed could also be factors (Ricke et al., 2020).

### Synbiotics

In May 2019, the ISAPP convened a panel of nutritionists, physiologists, and microbiologists to review the definition and scope of synbiotics. The panel updated the definition of a synbiotic to "a mixture comprising live microorganisms

and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host". The panel concluded that defining synbiotics as simply a mixture of probiotics and prebiotics could suppress the innovation of synbiotics that are designed to function cooperatively. Requiring that within this definition, 'host' microorganisms comprise both autochthonous (resident or colonizing the host) and allochthonous (externally applied, such as probiotics) microorganisms, either of which can be targeted for the substrate contained in the synbiotic (Swanson et al., 2020).

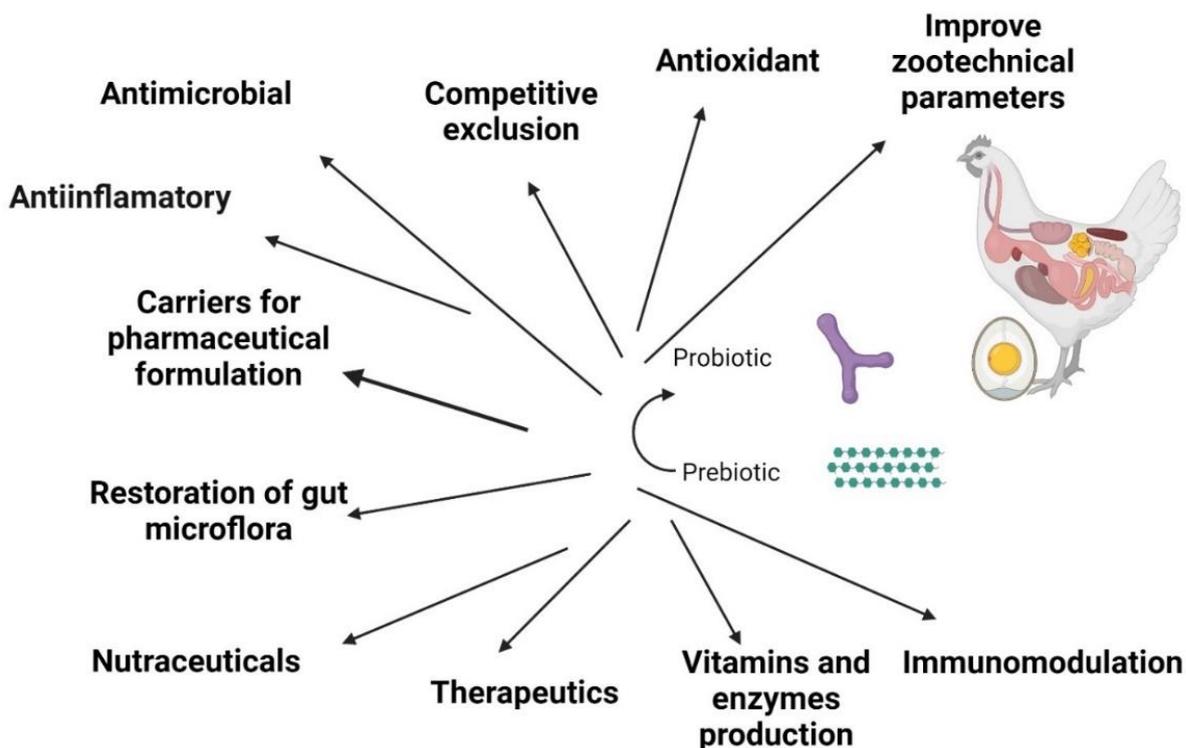
A synbiotic can be applied to intestinal or extra-intestinal microbial ecosystems and might be formulated into products fitting an array of regulatory categories (such as foods, non-foods, feeds, drugs, or nutritional supplements). Two categories of synbiotics are recognized. A 'complementary synbiotic' is a synbiotic composed of a probiotic combined with a prebiotic, which is designed to target autochthonous microorganisms. A 'synergistic synbiotic' is a synbiotic in which the substrate is designed to be selectively utilized by the co-administered microorganism(s) (Swanson et al., 2020). Because they wouldn't need responder strains to work, these synergistic synbiotics may be able to function even in prebiotic nonresponders. Moreover, adding a selective fermentable substrate provides a resource opportunity that raises the partner organism's competitive fitness and may lengthen its persistence (Krumbeck et al., 2016).

Thus, when carefully designed, synbiotics could offer a useful tactic to improve the durability and metabolic activity of particular advantageous probiotic strains. The probiotic components of the most often used synbiotic combinations are lactobacilli and bifidobacteria, whereas the prebiotic components include oligosaccharides, inulin, or fibers. Notwithstanding the apparent benefits of these feed additives, the precise way in which these synbiotics are made can have a big impact on how beneficial they may be (Krumbeck et al., 2016).

Figure 1 summarizes the many functions of synbiotics on the physiology of the digestive system. In chickens, synbiotics can be supplemented in feed/ water or injected *in ovo* to expedite colonization of the gut by beneficial bacteria. A hint of their *in vivo* biological potential can be found in the *in vitro* selection of synbiotics.

Both synbiotics can improve the general condition of the organisms, which are characterized by high production metrics and low mortality. Nonetheless, the synbiotic

composition influences the spleen's and cecal tonsils' gene expression, as well as the GIT's microbiota (Dunislawska et al., 2017).



**Figure 1:** The role of synbiotic on poultry.

### Synbiotic in poultry

Synbiotics have a beneficial effect on the performance parameters of chickens (daily cumulative mortality rate, feed conversion ratio, and the European Production Efficiency Factor), increase the count of beneficial bacteria (*Bifidobacterium* spp. and *Lactobacillus* spp.), and restrict the growth of potential pathogens in the GIT (*Clostridium* spp. and *Escherichia coli*). Also, synbiotics caused an increase in the concentration of lactic acid and short-chain fatty acid (SCFA) and a decrease in the concentration of branched-chain fatty acid (BCFA) in the broiler's excreta (Śliżewska et al., 2020).

Furthermore, the synbiotic PoultryStar® (Biomim; *Enterococcus faecium*, *Limosilactobacillus reuteri*, *Pediococcus acidilactici*, and *Bifidobacterium animalis* and FOS) can be used as an effective feed additive to improve productive performance. This synbiotic also has a beneficial effect on meat quality (increase dressing, breast, and leg percentages and decrease abdominal fat percentage), antioxidant capacity, and ammonia reduction,

as well as decreased microbial populations (*E. coli*, *Salmonella* spp., and *Shigella* spp.) (Abdel-Wareth et al., 2019; Hu et al., 2022). During heat stress, this synbiotic improves multiple indices of leg health, resulting in an improvement in locomotion ability (Yan et al., 2019; Hu et al., 2022). On the other hand, Song et al. (2022) investigated how a synbiotic made of FOS and microencapsulated *Lactiplantibacillus plantarum* affected broiler growth, immunological and antioxidant indices, and calcium and phosphorus digestibility. Its advantages for growth, immunological and antioxidant indices, and calcium and phosphorus digestibility suggest that it could potentially replace antibiotics in broiler diets.

### Postbiotics

Postbiotic is a term derived from the Greek for 'post', meaning after, and 'bios', meaning life. Further, the 'biotic' family of terms (probiotics, prebiotics, synbiotics, and postbiotics) coalesces around microbes (or their substrates). Therefore, the term postbiotic appropriately refers to substances derived after the microorganisms are

no longer alive or, in other words, inanimate, dead, or inactivated (Vinderola et al., 2022).

In 2019, the ISAPP convened a panel of experts specializing in nutrition, microbial physiology, gastroenterology, pediatrics, food science, and microbiology to review the definition and scope of postbiotics. The panel defined a postbiotic as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”. Inactivated microbial cells or cell components, with or without metabolites that support the reported health benefits, are necessary constituents of effective postbiotics (Salminen et al., 2021a). Published criticism of the definition raised concerns about alignment with previous definitions as well as queries on terminology (Aguilar-Toalá et al., 2021), to which a reply was published (Salminen et al., 2021b).

According to the Oxford etymology dictionary, inanimate means “without vital force, or having lost life.” For all practical purposes, ‘non-viable’ can be used as an appropriate synonym. As long as the microbe or microbes are identified to the strain level, the preparation process is sufficiently detailed, and the preparation's safety and efficacy are proven in appropriately conducted studies in the intended host, any microorganism or a combination of it can be utilized to create a postbiotic. Furthermore, bacteria that are pathogenic when alive could be employed to create a postbiotic. Cell biomass in the form of broken or fragmented cells is referred to as “components.” The term “components” refers to the understanding that different large molecular weight structures and sub-structures, such as peptidoglycans, teichoic acids, lipids found in microbial cell walls, and cell membranes, make up microorganisms. Numerous cellular constituents are recognized to possess immunogenic properties, suggesting their potential significance in conferring health advantages. Since cellular biomass (components) can only originate from live cells, they can only be referred to as “after-life” or postbiotic materials. Flow cytometry is a useful tool for measuring inanimate intact bacteria because it can differentiate between live, dead, and injured cells. Large molecular weight cellular components are challenging to measure. Hence, a proxy, such as total biomass, may need to be employed. Among other methods, mass spectrometry and HPLC can be used to quantify

any potential metabolites (Vinderola et al., 2024).

Postbiotics are secreted by food-grade microorganisms or released after cell lysis in complex microbial cultures (cell-free supernatant, CFS), food, or intestine (Liang and Xin, 2023). Many existing postbiotics include inanimate strains belonging to established probiotic taxa within some genera of the family *Lactobacillaceae* or the genus *Bifidobacterium*. However, the probiotic microorganism that gradually loses cell viability over the shelf life of the food does not gradually become a postbiotic; it is simply a probiotic food that, if formulated properly, will deliver an efficacious dose of live cells until the end of its shelf life. Furthermore, vaccines and purified microbial metabolites are not postbiotics. On the other hand, postbiotics have an action site that extends beyond the gut. A host surface, such as the oral cavity, stomach, skin, urogenital tract, or nasal cavity, is where postbiotics must be delivered, but they do not include injections (Salminen et al., 2021a; Vinderola et al., 2022).

One important factor driving interest in postbiotics is their inherent stability, both during industrial processes and storage. Given that probiotics' microorganisms are no longer able to replicate and can no longer cause bacteremia or fungemia—risks that relate to probiotic administration but are incredibly rare—postbiotics should be expected to have a better safety profile than probiotics. On the other hand, postbiotics' safety cannot be inferred from the progenitor microorganism's safety profile alone. Gram-negative bacteria, for instance, can produce lipopolysaccharides that can cause toxic shock and sepsis. This is particularly true when endotoxin A, which is typically buried in the outer membrane of active bacteria, is liberated from dead bacteria. Before using any postbiotic, its safety for the intended usage must be evaluated. Food-grade microbes or species included in the regularly updated EFSA QPS lists may provide postbiotics with an easier time (Salminen et al., 2021a)

The identification of the progenitor strain or strains will almost likely determine the biological activity of a postbiotic product, but the process of inactivation may also have an impact (Vinderola et al., 2024). The criteria for preparation to qualify as a postbiotic included molecular characterization of the progenitor microorganisms (for example, fully annotated

genome sequence) to enable accurate identification and screening for potential genes of safety concern, detailed description of the inactivation procedure and the matrix, confirmation that inactivation has occurred, evidence of a health benefit in the host from a controlled high-quality trial, detailed description of the composition of the postbiotic preparation, and assessment of safety of the postbiotic preparation in the target host for the intended use. A postbiotic concept or framework unique to foods or dietary supplements containing postbiotics has not been developed by regulators. Postbiotic compositions with pharmaceutical or medical applications in mind are subject to certain sophisticated regulatory requirements (Salminen et al., 2021a).

While probiotics are live bacteria with dosage variability and standardization problems, postbiotics are not connected to these issues, though. Postbiotics have an extended shelf life and require less care in comparison to probiotics, which require a lower storage temperature. Compared to probiotics (which do not include bacterial lysates), postbiotic production technology and quantitative control are significantly faster and more accurate. When it comes to safety, postbiotics are less risky than probiotics. Additionally, postbiotics avoid the potential issues with probiotics, such as virulence factors and antibiotic resistance genes (Kaur et al., 2021).

### **Mechanisms of action of postbiotics**

Postbiotics can act in five different ways (Saeed et al., 2023): i) Indirectly modulate resident microbiota: Postbiotics may transform the microbiota, such as by quenching, carrying quorum sensing molecules, or having lactic acid, which is used by some microorganisms to produce butyrate and short-chain fatty acids, which are helpful in the microbiota. ii) Improvement in the function of the intestinal barrier: if a postbiotic preparation contains enough short-chain fatty acids, it may prevent lipopolysaccharide-induced disturbances and change how epithelial barriers operate. iii) Alteration by systemic and local immune responses: Immune-modulating actions are usually elicited at the systemic and local levels by molecular patterns linked to microorganisms that engage with specific host pattern recognition receptors of immune cells. These

receptors, which include C-type lectins, Toll-like receptors, and receptors of the nucleotide-binding oligomerization domain, oversee the control of immunological responses and cytokines. iv) Alteration of systemic metabolic response: Systemic metabolic reactions in postbiotics may be directly impacted by the enzymes and metabolites on and inside the surface of inactivated microorganisms. Bile acids influence the host's metabolic processes, including those involving lipids, xenobiotics, glucose, and energy metabolism. They also alter the shape of the microbiota population and interact with numerous receptors. v) Systemic signaling through the nervous system: metabolites in probiotic preparation, including short-chain fatty acids produced by microbes, release serotonin by activating enterochromaffin cells, which subsequently enter the bloodstream.

### **Postbiotics in poultry**

Postbiotics is a relatively new concept in animal nutrition. Recently, Saeed et al. (2023) reviewed the most recent research investigating the beneficial results of postbiotics in poultry and concluded that postbiotic compounds significantly increased poultry performance. They are regarded as immunostimulators, anti-inflammatory, antioxidants, and anti-microbial, as well as growth promoters in poultry. The cell wall components and cytoplasmic extracts of various *Lactobacilli* species, including *L. acidophilus*, *L. casei*, *L. fermentum*, *L. rhamnosus*, *L. paracasei*, *L. delbrueckii subsp. bulgaricus*, *L. gasseri*, *L. helveticus*, *L. reuteri*, *L. johnsonii*, and *Bacillus coagulans* were found to be highly effective postbiotics (Abd El-Ghany, 2020).

*In vitro*, the cell wall components and cytoplasmic extracts of *Lactobacilli* (*L. acidophilus*, *L. casei*, *L. delbrueckii subsp. bulgaricus*, *L. gasseri*, and *L. helveticus*) and *Bifidobacterium* sp. demonstrated the ability to stimulate immune cells and produce nitric oxide and cytokines (Tejada-Simon and Pestka, 1999). Johnson et al. (2019) described the mechanism of action of a postbiotic in the context of a *C. perfringens* challenge model. The postbiotic (organic acids produced from a consortium or cocktail containing the following strains: *Pediococcus acidilactici* NRRL B-67717, *Limosilactobacillus -ex-Lactobacillus- reuteri* NRRL B-67718, *Enterococcus faecium* NRRL B-67720, and *Lactobacillus acidophilus* NRRL B-67701)

improved lesion scores, *C. perfringens* counts and mortality in broiler chickens compared to challenge groups without the postbiotic, and it improved weight gain in the most severely challenged birds. The postbiotic predominantly affects the innate immune response and appears immunomodulatory, reducing the proinflammatory responses and generating a homeostatic-like response.

Humam et al. (2019) studied the effects of feeding different postbiotics (cell-free supernatant -CFS- of *Lactiplantibacillus* -ex-*Lactobacillus*-*plantarum* strains defined as RI11, RS5 and UL4) on growth performance, carcass yield, intestinal morphology, gut microbiota, immune status, and growth hormone receptor (GHR) and insulin-like growth factor 1 (IGF-1) gene expression in broiler chicks under heat stress. They demonstrated that, although carcass parameters were not affected by the postbiotic-supplemented diet, this supplementation improved villi height significantly in the duodenum, jejunum, and ileum and increased the villus height to crypt depth ratio of duodenum and ileum, while decreased *Enterobacteriaceae* and *E. coli* counts and caecal pH. Furthermore, birds fed RI11 diets increased final body weight, total weight gain, and average daily gain, caecum total bacteria and *Lactobacillus* count, plasma immunoglobulins M (IgM) and IgG, and decreased *Salmonella* spp count. On the other hand, Loh et al. (2010) studied the effects of feeding different dosages of metabolite combination of *L. plantarum* RS5, RI11, RG14, and RG11 strains (Com3456) on the performance of broiler chickens. They found that supplementation of Com3456 with different dosages improved growth performance, reduced *Enterobacteriaceae*, and increased lactic acid bacteria count. Increased villi height of the small intestine and fecal volatile fatty acid concentration, being 0.2%, is an optimum level to be included in the diets of broilers to replace antibiotic growth promoters.

Postbiotics derived from yeast fermentation (Original XPC™, Diamond V, Cedar Rapids, IA) inhibited the shedding, downstream virulence, and antibiotic resistance of *Salmonella* spp. (Feye et al., 2016), and reduced heat stress indicators (plasma corticosterone and heterophil/lymphocyte ratio) under heat stress or no heat stress conditions (Price et al., 2016),

and it may influence cecal microbiota fermentation and has the potential to reduce *Salmonella* spp in the cecum in broilers (Rubinelli et al., 2016). Furthermore, it reduced *Salmonella* ser. Enteritidis in ceca of commercial laying hens (Gingerich et al., 2021). Also, this product could be used in conjunction with a live coccidiosis vaccine to increase growth rate and improve feed conversion of broilers (Roto et al. 2017), but it did not have any significant impact on the microbial and phylogenetic diversity of cecal microbiota (Park et al., 2017).

*Lactobacillus animalis* CFS improved performance and promoted quails' health by modulating gut microbiota. They increased LAB and decreased *E. coli* from intestinal microbiota, but they did not produce any modification in the level of organic acids (acetic, lactic, and propionic) in gut digesta (Kareem et al., 2020).

Postbiotics and prebiotics can be used together. Kareem et al. (2016) demonstrated that postbiotics (CFS of *Lactobacillus plantarum* RG14 and *L. plantarum* RI11) and inulin (prebiotic) combinations had beneficial effects on total body weight, feed efficiency, mucosa architecture, and liver insulin-like growth factor 1 (IGF1) and growth hormone receptor (GHR) mRNA expressions in broiler chickens. Furthermore, supplementation of combinations of postbiotic, only composed by CFS of *Lactobacillus plantarum* RG14, and inulin in the diet of broiler chickens improved growth performance, population of total bacteria and beneficial bacteria, reduced the population of *Enterobacteria* and *E. coli*, and increased acetic acid concentration with associated alterations in ileal cytokine expression. Although treatments with 0.15% and 0.45% RG14 displayed the best results, especially in terms of growth performance, cecal total bacteria, and cytokine expression, economically, postbiotic RG14 supplementation, 0.15%+1.0% inulin was preferred to be used as an optimal level for replacements for AGP in the poultry industry (Kareem et al., 2017). On the other hand, amoxicillin and Culbac (an aqueous postbiotic composed of a nonviable *Lactobacillus acidophilus* species fermentation product; TransAgra International Inc., Storm Lake, Iowa) produced the best ameliorating impact on necrotic enteritis, caused by *C. perfringens*, in broiler chicks. However, the combination feed and water treatment of Culbac produced more encouraging results for reducing necrotic

enteritis in terms of improving the hepatic health and humoral immune response of broiler chickens experimentally infected with *C. perfringens*, as compared to probiotics and antibiotics (Abd El-Ghany et al., 2022).

## Conclusions

Probiotics, prebiotics, synbiotics, and postbiotics are strategies that can be used to replace antibiotics as growth promoters and preventive uses in poultry production. Many products only have *in vitro* studies. However, the live or dead microorganism, substrate, or component for/of them should be very well characterized by *in vitro* and *in vivo* (poultry) studies if the product wants to be sold commercially. Also, if the probiotic microorganism is alive, it should be necessary to study the presence of antibiotic-resistance genes (phenotypic and genotypic studies), especially those encoded by plasmids that can be transferred between organisms. The presence of some of these genes causes the microorganism to be ruled out as a probiotic.

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