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Review

Effects of prebiotics and precision biotics on performance, animal welfare and environmental impact. A review

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A R T I C L E I N F O

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A B S T R A C T

This review aims to analyze the recent studies about prebiotics and precision biotics, as alternatives to animal growth promoters. These substances improve intestinal health, growth performance and poultry environmental impact. Prebiotics are insoluble fibers, that have no nutritive value, but they promote the growth of positive bacteria, increase the nutrients absorption and modulate the immune response. Instead, precision biotics are carbohydrates with glycosidic linkages, which interact with gut bacteria metabolism, reducing the excretion of nitrogen and consequentially, the poultry environmental impact. In the last years, different studies were published in this field, and for this reason, it is necessary to organize the results found. It was shown that mannan-oligosaccharides and β-glucans increase ileal nutrient digestibility, nitrogen retention and antibodies titers. Inulin, arabinoxylans-derived oligosaccharides, and galacto-oligosaccharides improved intestinal morphology, arranging for a larger absorption surface area. It was reported that prebiotics enhance the colonization of positive bacteria and can reduce the count of *Campylobacter* colonies. Furthermore, xylo-oligosaccharides are often used in animal feed, due to their ability to form organic acids, which decompose noxious substances, improving litter quality, and consequentially, reducing the environmental impact. Litter quality is a relevant aspect for ammonia emissions and for animal welfare. Whether the litter quality is poor, footpad dermatitis increase, worsening animal welfare and increasing nitrogen emissions to air. Precision biotics select metabolic pathways to modulate amino acid degradation, reintegrating the nitrogen discarded, and reducing the ammonia level in litter. It was also reported an improvement of growth performance and a better animal welfare. In conclusion, prebiotics and precision biotics can have positive effects on animal performance and welfare, and they can be a new strategy to reduce the environmental impact of chickens' farms.

1. Introduction

Aviculture is the most efficient and productive livestock sector. In fact, it is a widespread industry with a global production of 138.8 million tons of meat in 2022, rising by 0,6 % from 2021, the slowest pace of growth recorded, due to the high costs of feed and energy (FAO, 2022). A continuously raising human population is increasing the global demand for food. People in countries with a higher socioeconomic status tend to prefer meat and high-value foods rather than grains or vegetables. Their preference for chicken as a source of proteins has grown by 70 % over the last three decades (Kalia et al., 2022). In fact, the increasing demand of poultry meat led to an enhancement of broilers' growth rate, feed efficiency, size of breast muscle and higher standards of meat to improve its functional properties and its taste (Petracci and Cavani, 2012). The high demand and the efficiency of intensive poultry husbandry led to a significant environmental impact. Poultry litter and manure have an effect on global greenhouse gas emissions, as well as animal and human health. In fact, they can contain pesticide residues, pathogen microorganisms, and other pollutants, which can lead to air, soil and water contamination (Gržinić et al., 2023). In addition, nowadays people pay more attention to animal welfare and environmentally friendly products. Animal conditions in breeding and environmental impact of livestock systems are the first motivations guiding consumers in their purchasing choices. In fact, the demand for sustainable products is increasing: more than 66 % of people worldwide are willing to pay for sustainable offerings (Mazzocchi et al., 2022).

In the past 70 years, antibiotics were widely used in sub-doses in animal diets, not only to control infectious diseases, but also as animal growth promoters (AGPs), to improve animal growth performance and feed efficiency, and to have healthier and stronger animals (Al-

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Khalaifah, 2018). Their mode of action is still not fully understood: they modify the gut microbiota of animals, because their target are Gram-positive bacteria (for example *Clostridium* spp.) associated to lower performance and poorer health. Moreover, they increase the thickness of the intestinal wall, enhance absorption, and allow a better utilization of nutrients (Kleyn, 2013). The wider use of these substances had contributed to the development of resistant bacteria, such as microorganisms against which drugs to treat infections have no effect. In addition, these bacteria are spread into the soil, where they can survive and contaminate the environment (Mazhar et al., 2021). For human health, they represent a potential risk, so the European Union banned the use of antibiotics as growth promoters in animal feed in 2006. The exclusion of AGPs led to many problems in the production, such as the increase of feed conversion rate (FCR) and the increase of animal diseases, so animal welfare got worse. Thanks to the research, they were replaced by some dietary supplements, such as probiotics, prebiotics, and modulators of microbial metabolism (MMMs), that are claimed to enhance growth rate and to modulate positively the immune response (Al-Khalaifah, 2018; Puvača et al., 2020). So, it begs the question: can these additives be considered good substitutes for AGPs? Before answering this question, it is necessary to understand how these substances work and what is their target.

Nowadays, intestinal health is one of the most discussed issues in animal world, thanks to its significance in many biological processes. Maintaining a balance of good gut health is critical for optimal growth and feed efficiency (Vasanthakumari et al., 2023). A healthy intestine allows a better utilization and absorption of nutrients and consequently, a lower excretion of metabolites (Moita and Kim, 2022). Its preservation is attributed to the microorganisms that inhabit the gastrointestinal tract, known as microbiota, which closely and intensively interact with the host (Yalçın et al., 2023). The most complex microbial community in chickens' gut is the one in cecum, consisting of the phyla of *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria* (Mancabelli et al., 2016). These microorganisms have different functions:

- Exchange of nutrients;
- Modulation of immune system;
- Exclusion of pathogens, occupying certain ecological niches or producing lactic acid from fermentation of carbohydrates, which lowers the intestinal pH and inhibits the growth of certain pathogen bacteria, such as *Escherichia coli* and *Salmonella Typhimurium* (Pan and Yu, 2013).

In diets, there are both digestible carbohydrates that are absorbed in the proximal gut, and indigestible carbohydrates, which are hydrolyzed by bacteria in polysaccharides, oligosaccharides, and disaccharides, obtaining short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. These compounds are absorbed in cecum, and are used by animals as energy source, because they can enter in different metabolic pathways, for example butyrate is a source of energy for epithelial cells. Furthermore, SCFAs can regulate intestinal blood flow, stimulate enterocyte growth and proliferation, and mucin production. In addition, there is evidence that cecal bacteria can catabolize uric acid, which comes from retrograde peristalsis in rectum, to ammonia, that is absorbed, and not excreted, by chickens and used to synthesize amino acids (Mancabelli et al., 2016; Clavijo and Flórez, 2018). Moreover, microbiota can modulate the immune system, due to commensal microorganisms that control the quantity of mediators secreted by immune system cells and they stimulate t-helper cells, even if mechanisms are not completely clear. The inner surface of avian gut is coated with layer of mucus, formed by a glycoprotein called mucin, secreted by goblet epithelial cells. This substance is a component of intestinal mucosal innate immune system, and it repels most bacteria, which cannot adhere to the intestinal barrier, so they cannot colonize gut. This is the first line defense against infections. Another defense is the antimicrobial peptides on the intestinal epithelial surface, such as β-defensins. They are small cationic peptides produced by macrophages and epithelial cells, that can disrupt cell membrane of pathogens and lead them to cell lysis (Mancabelli et al., 2016; Clavijo and Flórez, 2018). In conclusion, it is possible to affirm that gut microbiota is like an organ, which if functioning and properly developed, ensures intestinal health. In fact, it must be considered that enteric diseases are an important concern to chickens' farms, due to the loss of money related to the decreased performance and increased mortality. In the past decades, gut health was ensured by AGPs, but after the ban, different substances have been proposed, as already mentioned (Hajati and Rezaei, 2010). However, high stocking densities make gut health management difficult (Yalçın et al., 2023). Dirty litter, feeding practices and other farm management affect the composition of gut microbiota, because they can be a source of pathogen bacteria and they can negatively influence the immune defense of chickens. Instead, having an optimal gut microbiome (environment where microorganisms live in the gut) helps to manage stressful situations, as reported in many studies (Anadón et al., 2019).

Gibson and Roberfroid (1995) defined a prebiotic as "a non-digestive food ingredient that beneficially affects the host by stimulating selectively the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health". So, a prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). In fact, prebiotics are a non-digestible substances, that are used by bacteria for their fermentations with consequentially production of SCFAs, which lower pH and select commensal bacteria (e.g. *Bifidobacterium* spp. and *Lactobacillus* spp.) (Pourabedin and Zhao, 2015). The main prebiotics are usually insoluble fibers, isolated from plants, legumes, grains and cruciferous vegetables and fruits and, even if they have no nutritive value, they still have different functions, like the physical stimulation of intestine and the selection of microbiota composition, that is why are important to be included in monogastric diets. The most used prebiotics are non-starch polysaccharides (NSP) or oligosaccharides like xylo-oligosaccharides (XOS), fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS), and galacto-oligosaccharides (GOS) (Jahan et al., 2022). These compounds are composed by 3–10 monosaccharide units, which can be linear or branched and they are linked by α- or β-glycosidic bonds (Jahan et al., 2022). Due to the inability of broilers to hydrolyze these compounds, they reach cecum undigested, where are fermented by microbiota (Pourabedin and Zhao, 2015).

Instead, probiotics are a collection of live microorganisms, which confer a health benefit on gut's host, whether administered in adequate quantities. To be considered a probiotic, a microorganism must:

- be non-pathogenic;
- have the ability to adhere to epithelial cells;
- have the ability to colonize and reproduce in the host;
- have the ability to survive in the host;
- have the ability to survive along the gastrointestinal tract;
- resist gastric acidity and bile;
- produce metabolites that inhibit or kill pathogenic bacteria;
- be subjected to in vitro and in vivo tests demonstrating its benefits and efficacy.

It is essential that they stay alive under production and during storage conditions (Clavijo and Flórez, 2018). The bacterial genera commonly used as probiotics are *Bacillus*, *Lactobacillus*, *Enterococcus*, *Bifidobacterium* and *Streptococcus* (Khan et al., 2020). They have several benefits, such as the reduction and prevention of pathogenic bacteria, the increase of the digestive capacity and the stimulation of the intestinal epithelium, and the modulation of immunological activity (Clavijo and Flórez, 2018; Pan and Yu, 2013). However, the efficacy of probiotics depends on several factors, such as the composition of the mixture and their stability, the age at which they are administered, the origin of the microorganisms and the conditions of the environment in which chickens live (Clavijo and Flórez, 2018). Compared to prebiotics, probiotics are more expensive, the risks of unwanted effects in the host are higher, the production process and administration are more difficult to manage, and their effectiveness is more variable (Anadón et al., 2019; Clavijo and Flórez, 2018).

In addition to prebiotics and probiotics, there is a new category of additives, called microbiome metabolic modulators (MMMs), which can control metagenomic functions and to modulate the metabolic pathways of gut microorganisms. In this new category, precision biotics (PBs) are included: they are carbohydrates with glycosidic linkages, that can control the metabolism of gut bacteria and its metabolites, such as short-chain fatty acids production and nitrogen compounds, safeguarding the environmental impact. An example of PBs is tailored glycans, which have a chemical structure that regulate the abundance of genes that activate certain metabolic pathways, unlike prebiotics which modulate microbial taxa. In fact, microbiota's pathways are highly conserved across different species of bacteria, and this is an advantage, because taxonomic composition often varies significantly between chickens from different farms, due to the influence of environment. Moreover, by controlling metabolic pathways and consequentially their metabolites, these additives can promote beneficial outcomes to animals and to the environment, improving energy efficiency and reducing intestinal ammonia production (Blokker et al., 2022; Jacquier et al., 2022). This is very important, because nowadays sustainability is a discussed topic. Although this sector has been found to be relatively "environmentally friendly" compared with other livestock production, it still has an impact on global warming and on ground. Feed production and its transport (for example the importation of protein sources, such as soya) contribute about 70 % of emissions of the sector. Instead, the management of poultry manure contributes about 40–60 % of the ammonia emission of poultry industry, due to the decomposition of uric acid by some bacteria present in litter. While the problem of ruminants is the global emission of greenhouse gas, for chickens the main problem is ammonia (NH_3) emissions to air and nitrous oxide emissions, that contribute to global warming and nitrate leaching (Leinonen and Kyriazakis, 2016). Ammonia has a strong impact also on animal welfare because the exposure to NH_3 alter the trachea surface, causing cilia paralysis and mucus cannot be removed, exposing the lungs and airbags to pathogen bacteria in dust (e.g., septicemia *Escherichia coli*). Furthermore, long exposure to ammonia levels can cause inflammation on eyes, such as conjunctivitis, and corneal skin damage, reducing weight gain and growth performance (Swelum et al., 2021). In the last years, genetic selection has allowed a high feed efficiency and a better feed conversion rate (FCR), so a bird requires less feed to achieve the same slaughter weight. Greenhouse gas emissions from fossil fuels used in crops' production and emissions from animals are reduced thanks to the more efficiency of chickens in using nutrients' feed (Tallentire et al., 2016). However, the diet can be still improved to further reduce the environmental impact by increasing its digestibility adding enzymes (e.g., xylanase or β-glucanase that break down the non-starch polysaccharides and reduce the digesta viscosity (Pan and Yu, 2013)) or these PBs, which can lower excretion of nitrogen (Leinonen and Kyriazakis, 2016).

In bibliography, there are many studies about the addition of prebiotics and precision biotics in broilers' diet. For this reason, it is necessary to organize the information published in this field. This review aims to analyze the results obtained in the recent studies about the effects of these substances, on chickens' performance, welfare, and environmental impact.

2. Methodology

This review analyzes scientific studies on the effects on growth performance, welfare, and environmental impact, reported by the use of prebiotics and precision biotics in broilers' diet. An initial literature search was performed using Scopus, as database. The keywords used were "prebiotic* AND broiler*", selecting the timeframe from 2019 to 2023 and reducing the subject area to "environmental science" and "agricultural and biological sciences". Other criteria applied were:

- the language: a study for being included had to be written in English;
- the access: all the studies used were open access.

At the moment of the search, 147 studies were found: papers about synbiotics, phytobiotics, plant extracts, microelements and experiments on laying hens were excluded, because the aim of this review was to highlight the effects of prebiotics and precision biotics, as new technology, on broilers' performance, welfare, and environmental impact. A second search was performed using the words "precision biotic* AND broiler*": only four studies were found. A third search was conducted using the words "NH_3 emission AND NH_3 toxicity AND management" with the same criteria as before: four studies were found, but only one was used.

3. Results

The literature analyzed showed that prebiotics can reduce stress for high densities or high temperatures in broilers, thanks to the improvement of intestinal ecosystem and morphology, resulting in a better animal welfare. Even in small quantities, prebiotics stimulate the production of mucus by goblet cells, which increase intestinal barrier integrity that allows a stronger immune defense, protecting against the negative effects of stress (Gül et al., 2022; Sugiharto, 2022). It has been shown that heat stress causes an intestinal dysbiosis, oxidative status and compromises the physiological stress response. Prebiotics alleviate these negative impacts, limiting losses and restoring growth performance, because they maintain gut eubiosis (Awad et al., 2021). In fact, one of the first consequences of heat stress is the intestinal hyperpermeability, which causes a major risk of pathogens colonization (Ringseis and Eder, 2022). However, management practices and the husbandry environment influence the effectiveness of prebiotics. If large numbers of bacterial colonies are present in the environment, prebiotics will not be the solution (Zhu et al., 2021).

Another relevant factor is composition of the diet. Usually, commercial diets contain only 2–3 % of crude fiber. It has been demonstrated that increasing the level of cellulose at 3–5 % in broilers diets can improve nutrient utilization. Dietary fiber increases pancreas enzymes and reverse peristalsis, which causes bile salts to reach the gizzard, where the bolus is mixed with gastric secretions, leading to an increase in nutrient digestibility. These results in an improved fat emulsification, reducing the potential of fat droplets to coat nutrients, and therefore, nutrients are more readily hydrolyzed and absorbed, but this process is influenced heavily by the source of fiber (Tejeda and Kim, 2021).

Exploring in detail the different types of prebiotics, data obtained from the selected studies are summarized in the table below (Table 1).

3.1. Yeast cell wall derivatives

Mannan-oligosaccharides and β-glucans are derived from yeast cell walls of *Saccharomyces cerevisiae*. It is widely known and reported that they improve growth performance, regulate intestinal microbiota, and stimulate immune responses. In fact, MOS are often used in stressful situations because they promote the growth of *Lactobacillus* and *Bifidobacterium* species in the cecum and they bind pathogens, preventing their colonization, thanks to their affinity ligand. MOS stimulate innate immune-modulatory activities through their cell surface receptor that recognizes host glycoproteins and microbial glycans or via mannose-binding-lectins that trigger an inflammatory response by initiating a cascade of cytokine expression. Also, β-glucans are immunomodulators and they enhance the proliferation of lymphocytes (Pourabedin and Zhao, 2015; Teng et al., 2021; Jahan et al., 2022). In a study of Froebel et al. (2019), refined functional carbohydrates (RFC), were used to compare the effects of prebiotics to antibiotics. RFC are a mix of mannan-oligosaccharides, ß-glucans, and D-mannose, derived from the

Table 1
A list of studies since 2019 describing the effects of different prebiotics on performance, gastrointestinal and immune system of broiler chickens supplemented at different levels in diets.

Hybrid	Prebiotic	Dose	Effects on performance	Effects on gastrointestinal system	Effects on immune system	Authors
Cobb	Refined functional carbohydrates (RFC)	50 g/t and 100 g/t	Better BW and ADG. No effects on FCR	Reduced the count of *Campylobacter* spp.	N.A.	Froebel et al., 2019
Cobb	MOS + β-glucans	0.04 % MOS and 0.002 % beta-glucan	Increased BW and BWG in the starter period and β-glucans until day 28.	Significantly increased villus height	N.A.	Po-Yun Teng et al., 2021
Ross 308	β-Glucans	0, 50, 100, and 150 mg/kg of diet	FI increased in groups receiving 50 and 100 mg/kg	Longer villi and a shorter crypt depth	Hypolipidemic impact and improved hormonal profile	Amer et al., 2022
Ross 308	MOS + phytase	100 g/t of diet	Increased BWG and better FCR	Jejunal villus length and positive changes in microbial population and higher level of calcium in blood	Increased serum concentration of calcium	Karimian and Rezaeipour, 2020
Cobb	FOS and MOS	5 g/kg	No significant effects on BW, BWG, feed efficiency	N.A.	Higher cellular response and increased titers of antibodies (IgY and IgA)	H. Al-Khalaifa et al., 2019
Ross 308	Hydrolysed yeast and yeast cell wall (70 % MOS)	1 kg/t	Improved average daily weight gain (ADWG) and FCR in the starter period	Increased lactobacillus population and reduced coliforms population, especially *E. coli*	Higher titers of antibodies than control diets, but lower than synbiotic	Ghasemi et al., 2020
Unspecified	MOS	0, 50 and 100 g/kg of feed	Increased feed intake (FI), BWG and FCR	Increased villus height, crypt depth and goblet cells count	N.A.	Chand et al., 2019
Ross 308	Yeast cell wall of *S. cerevisiae* in groups challenged with *S. Typhimurium* and *C. perfrigens*	0,5 g/kg	Increased FCR. No differences in daily feed intake	Increased villus surface area, reduced colonies of *S. Typhimurium* and increased lactobacilli	N.A.	Alkhulaifi et al., 2022
Cobb 500	XOS and xylanase	50 g/t and 100 g/t	Xylanase and XOS had no statistically interaction effects on growth parameters	No effects on VH and CD	The immune markers of T-cell and B-cell were not different across treatments	Singh et al., 2021
Ross 308	XOS and xylanase	0,25 g/kg and 1,0 g/kg	Reduced FI, improved FCR, but XOS had no effect on BWG	Increasing concentration of arabinose, fructose, and galactose in ileum	N.A.	Craig et al., 2020
Ross 308	Beta 1–4, *endo*-xylanase producing oligosaccharides in situ	0, 45,000 U/kg, and 90,000 U/kg	Improved the feed efficiency, ileal nutrient digestibility, and ileal digestible energy	Increased the abundance of commensal bacteria such as *Lactobacillus*	N.A.	Vasanthakumari et al., 2023
Ross 308	XOS and gamma-irradiated astragalus polysaccharides	100 mg/kg of XOS and 600 mg/kg of IAPS	N.A.	Decrease of microbiota richness and an increase of *Ruminococcaceae*	Higher serum lysozyme activity and higher IgA-producing cells number	Wang et al., 2022
Ross 308	AXOS	0,50 % of diet	No impact on FI, FCR and BWG	Stimulation of fiber degrading capacity of the existent young microbiota	N.A.	Bautil et al., 2020
Dahen broiler chicken	XOS and GOS	1 % of diet	No statistically significant differences in average body weight, breast muscle percentage, thigh muscle percentage, and abdominal fat percentage	Altered the contents of caecal metabolites related to flavor substances	N.A.	Yang et al., 2022
Ross 308	Inulin + wheat bran	2 % of inulin +10 % wheat bran	BWG, FI and FCR not influenced	Greater villus height	N.A.	Li et al., 2019
Tegel	Inulin	10 g/kg, 20 g/kg and 40 g/kg	No effect on BW, FCR and FI	Increased *Bifidobacterium* strains	Increased IgM	Xia et al., 2019
Ross 308	Inulin, yeast product and wheat bran	5 g/kg, 0,05 g/kg and 3/6 % of diet	Wheat bran diet added with xylanase improved BW, BWG and FCR	N.A.	N.A.	Such et al., 2023

N.A. = not available.

cell wall of *Saccharomyces cerevisiae*. The authors made 6 experimental treatment groups:

- Control diet;
- Antibiotic diet (bacitracin methylene disalicylate);
- High-dose (100 g/t) of prebiotic RFC in feed;
- Low-dose (50 g/t) of prebiotic in feed;
- High-dose (100 g/t) of prebiotic RFC in feed and via drinking water;
- Low-dose (50 g/t) of prebiotic in feed and via drinking water.

They found that body weight (BW) and average daily growth (ADG) were greatest when broilers were fed the high level of prebiotics (100 g/t), as compared to the low prebiotics and control diet, but treatments had no effect on feed conversion rate. Furthermore, the high dose allows the reduction of count of *Campylobacter* spp., suggesting the ability of RFC to inhibit adhesion of pathogens to the gastrointestinal mucosa, reducing its prevalence in litter, probably due to the presence of MOS. The improved growth performances observed in prebiotic treated groups were attributed to the increase of ileal nutrient digestibility, nitrogen retention, villus height, and colonization of positive bacteria, such as *Bifidobacterium* spp. and *Lactobacillus* spp. However, the authors reported that there was not a significant increase in performance with the addition of the prebiotic in drinking water.

Other authors hypothesized that MOS and β-glucans might positively interact to improve growth performance and intestinal health in broilers. They made 4 treatment groups: a control, a MOS treatment, a β-glucan treatment, and a combination group with the two prebiotics. The supplementation of MOS or β-glucans increased body weight and body weight gain (BWG) of animals in the first 2 weeks. Moreover, β-glucans further improved BW and BWG until day 28. In this case, the authors did not report a statistically significant effect of MOS on performance growth and there was not a significant interaction between MOS and β-glucans. However, the combination of MOS and β-glucans improved intestinal morphology, significantly increasing villus height and presented a trend of upregulation of immune responses in the ileum and cecal tonsil (Teng et al., 2021). β-Glucans are already known for enhancing intestinal health in poultry exposed to a bacterial challenge, because they promote macrophage activity, and have an anti-inflammatory effect, due to macrophages that detect them, producing pro-inflammatory cytokines, such as IL1 and tumor necrosis factor-alpha. Their effect on immune response and on intestinal health can improve general welfare of broilers. In a study of Amer et al. (2022) animals were fed on four experimental diets, which varied only for the content of β-glucans (0, 50, 100, and 150 mg/kg of diet, respectively). During the starter period, no marked variations were detected between the groups, concerning the BW, BWG, feed intake (FI), and FCR. Through the growth period, FI increased in the group receiving 100 mg/kg of β-glucans, but the in general performance was not influenced by the prebiotic; only total feed intake was increased in groups receiving 50 and 100 mg/kg of β-glucans. This means that broilers fed the prebiotic eat more, but do not convert this feed into weight gain, due to the low energetic contribution of glucans, but not all the studies agree. Other factors must be considered, like the composition of glucans, dosage, purity, species, strains. In this study, it was showed an improvement of intestinal histomorphology of broilers receiving supplemented diets: long villi and a short crypt depth. This effect could allow a better nutrient utilization and absorption, but growth performance was not influenced, probably because the energy contribution was redirected to other pathways. The authors demonstrated that β-glucans improve the intestinal barrier mechanism and maintain the mucous membrane integrity by promoting the development of neurotransmitters. Furthermore, prebiotics improved gut motility and promoted microbiota diversity. On the other hand, it is reported that the inclusion of these prebiotics, that improve intestinal morphology and increase surface absorption, can alter the enzyme activities, for example the exogenous phytase supplementation. The poor gut availability of phosphorous, its effects on environmental pollution and the interference with macrominerals and protein absorption, led research to try to integrate an exogenous microbial phytase to hydrolyze dietary phytate. If added to the diet, phytase supplementation improves gut health and nutrient utilization, thanks to the better digestion and absorption of amino acids and minerals (Karimian and Rezaeipour, 2020). In 2020, Karimian and Rezaejpour studied the effect of combined phytase and MOS in broilers' diet. They found that this combination can increase body weight gain, can improve FCR and increase the level of calcium in blood, due to the breakdown of the structure of calcium-bound phytate units. However, more studies in this field are needed.

Instead, Al-Khalaifa et al. (2019) did not find significant effects on performance in broilers fed on MOS or FOS, compared to chickens with a diet supplemented with a probiotic. Feed consumption was significantly higher in groups receiving MOS than in those fed with the probiotic. The best result in this study was about the immune response, because animals fed MOS and FOS showed higher cellular response and an increase of antibodies titers (IgY and IgA) than other treatments (Al-Khalaifa et al., 2019).

In fact, not all the authors obtained the same linear results. Some studies found better performance by adding prebiotics only in the starter period of broilers, probably due to the unstable microbiota in the early days of chickens. In this period, the stimulation of bacteria's growth may improve performance, increasing *Lactobacillus* population and reducing *Coliforms* like *E. coli* (Ghasemi et al., 2020). Ghasemi et al. (2020) hypothesized that the action of prebiotic would be more effective if chickens were reared under stressful conditions, like high flocking density or the presence of pathogenic bacteria, such *Salmonella*, *E. coli* or *Clostridium*. *Salmonella Typhimurium* is a gram-negative infectious bacterium, known for decreasing performance, causing important economic losses. In addition, it is associated with one of the most relevant foodborne diseases in humans, due to its high resistance to antimicrobials. Instead, *Clostridium perfringens* is a gram-positive, spore-forming pathogenic bacterium, that induce the necrotic enteritis, which represents the 30 % of mortality in broilers and a loss of money too, basically due to the costs of treatments and the reductions in growth performance (Alkhulaifi et al., 2022). A study was conducted to determine whether losses from these pathogens could be limited by adding a prebiotic derived from yeast cell wall (YCW). Three hundred and sixty one-day-old chickens were divided into 5 groups:

- control group without neither bacterial challenges nor feed additives;
- a group infected with *S. Typhimurium*;
- a group challenged with *C. perfringens*;
- *S. Typhimurium* group with dietary supplementation of YCW;
- *C. perfringens* group with dietary supplementation of YCW.

The challenged groups with the supplementation of YCW had a better FCR compared with the unsupplemented challenged groups, especially the group infected with *S. Typhimurium* + YCW, that reached the same level of the control. However, there was no difference in daily feed intake among the groups. Regarding the effects on intestinal morphology, the villus surface area was more extended in groups challenged, but with the yeast's supplementation compared to control group. Furthermore, dietary supplementation with YCW in *S. Typhimurium* group lowered the *S. Typhimurium* colonies and increased *Lactobacillus* population. In conclusion, in this study the supplementation with YCW allowed a better restoration of growth performance and had a trophic influence on broilers' gut development, as demonstrated by the increased ileum villi height and surface area, limiting the well-known damages of these pathogens. These alterations in intestinal morphology of challenged animals were associated with enhanced growth performance compared to the unchallenged groups, thanks to a bigger surface area for nutrients' absorption (Alkhulaifi et al., 2022). Similar results with YCW were obtained by Barbalho et al. (2023), who inoculated in chickens *Clostridium perfringens* and *Eimeria acervuline*, a coccidium that affects birds and decreases performance.

3.2. Xylo-oligosaccharides

Another widely used and discussed prebiotic are xylo-oligosaccharides (XOS), which derived from the partial hydrolytic degradation of lignocellulosic materials contained in grains (Pourabedin and Zhao, 2015). They represent a carbon source for beneficial bacteria, increasing their proliferation and restricting pathogenic bacterial growth. XOS form organic acids, which decompose noxious substances, improving litter quality, and consequentially, environmental impact and animal welfare (Jahan et al., 2022). Litter quality is relevant aspect for ammonia emissions because the volatilization of NH_3 depends on pH, humidity level, ventilation rate, air velocity, and temperature. If litter pH is low, humidity and temperature not too high, and if there is a good ventilation and change of air, ammonia decreases, not only in air, but also in soil, where it causes eutrophication and acidification.

XOS can be obtained by adding exogenous enzymes in wheat-based diets, but it is also increasingly being used in corn-based diets. Adding enzymes to broilers' ration leads to countless gut health benefits, such as a better nutrients' utilization and subsequently an improvement of growth performance, thanks to the increase of mucosal surface area for absorption, that allows a better access of digestive enzymes to nutrients. Exogenous enzymes can also reduce the intestinal digesta viscosity and increase the fermentable substrates for microbiota (Singh et al., 2021). In addition, carbohydrases hydrolyze NSP, break them down into smaller oligosaccharides and release encapsulated nutrients that would not be used otherwise, like xylanase with XOS (Craig et al., 2020). To verify the interaction between xylanase and XOS on growth performance and gut health, Singh et al. (2021) divided 288 one-day-old chicks in three treatments with different levels of xylanase and XOS (0, 50 and 100 g/t). The authors found that the combination of xylanase and XOS had no effects on performance, but the increasing level of xylanase increased the average daily feed intake (ADFI). Moreover, ADG and final body weight gain (FBWG) were higher in diets containing xylanase, but the difference was not statistically significant. Ileal mucosa, villus height and crypts depth were not affected by treatments. Neither the immune markers of T-cell and B-cell. It was observed that both XOS and xylanase increased the production of acetate in the ceca, and it indicates their influence on modulating the fermentation characteristics of cecal microbiota, because they represent a selective substrate. On the other hand, Vasanthakumari et al. (2023) reported more beneficial effects of adding xylanase enzyme, such us lower intestinal digesta viscosity and an improved nutrient digestion, resulting in better gut health, due to the reduced availability of undigested nutrients for the growth of harmful bacteria. This enzyme produced oligosaccharides in situ with a prebiotic effect that could modulate the gut microbiome. Authors reported an improved feed efficiency, a better ileal nutrient digestibility, and higher abundance of commensal bacteria, such as *Lactobacillus* spp. and lower abundance of pathogen bacteria, such as *Escherichia coli* and *Shigella*.

Also, Craig et al. (2020) set up a study to investigate the effect of supplementing wheat-based diets, deficient in energy and protein, with xylanase or XOS on growth performance and the concentration of NSP hydrolysis products in the ileum. Protein sources are expensive, and often are inserted at excessive doses to stimulate growth, but some are lost and form uric acid excreted as ammonia. For this reason, nowadays an ideal protein is being studied, to achieve the exact balance of amino acids, not excess or scarce, to minimize nitrogen excretion (Swelum et al., 2021). In this study, five hundred Ross 308 one-day-old were allocated and divided in five treatments:

- the control;
- control plus xylanase 16,000 BXU/kg;
- control plus xylanase 32,000 BXU/kg;
- control plus purified XOS 0,25 g/kg;
- control plus purified XOS 1,0 g/kg.

The broilers in the study performed below breed standards, with a low energy and protein level to see if they could compensate for the deficit. The authors found that xylanase had a better effect on growth performance, attributing these results to the generation of prebiotic oligosaccharides in situ, during NSP hydrolysis caused by xylanase. The creation of prebiotics can provide additional benefits to the use of xylanases. The enzyme's supplementation improved BWG, FI and FCR. The authors attributed the results to the mechanism of action of xylanase, which releases the trapped nutrients and reduces digesta viscosity. In addition, when xylanase or XOS were added into the diet, feed intake reduced, resulting in decrease in FCR and an improvement of efficiency. In this study, XOS reduced feed intake and improved FCR, but had no effect on body weight gain, because this prebiotic involves the modulation of gut microflora and the increase of gut integrity. In fact, it was demonstrated the increase of SCFA, stimulating *Bifidobacterium* spp. and *Lactobacillus* spp. and discouraging the colonization of pathogenic bacteria. However, they found an unexpected result in the negative effect of XOS supplementation on IDE (ileal digestible energy) and no effect on nitrogen digestibility, but it was left without explanations. Investigating the NSP in ileum, it was found an increasing concentration of arabinose, fructose, and galactose in response to the addition of xylanase. These substances may have a beneficial effect on gut development and are linked to the effects of xylanase and XOS supplementation reported on growth performance. Consequently, it can be suggested that improvements in growth performance were partly driven by the production of in situ prebiotics following xylanase supplementation, but more studies are needed in this field.

Another interesting study investigated the individual and combined effects of XOS and gamma-irradiated astragalus polysaccharides (IAPS) on the immune response and intestinal microbiota composition of broilers. The intestinal mucosal immune system is a self-defense mechanism, which is composed of the gut-associated lymphoid tissue (GALT), including the Peyer's patches (PP), isolated lymphoid follicles (ILF) and mesenteric lymph node (MLN), and a large population of scattered immune cells. The GALT is the inductive and effector sites of the mucosal immune defense (Wang et al., 2022). The effects of XOS have already been mentioned, instead, gamma-irradiated astragalus polysaccharides are a physical modification product of native astragalus polysaccharides: it was reduced their molecular weight, which gives IAPS a higher immunomodulatory activity. In other studies, it was demonstrated that IAPS can improve growth performance and immunity of immunosuppressed broilers, so Wang et al. (2022) set up a study with 240 Ross 308 one-day-old chicks and allocated into five dietary treatments to see the effects of these prebiotics compared to an AGP (chlortetracycline):

- control diet;
- control diet supplemented with 50 mg/kg AGP;
- control diet supplemented with 100 mg/kg of XOS;
- control diet supplemented with 600 mg/kg of IAPS;
- control diet supplemented with 100 mg/kg XOS + 600 mg/kg IAPS.

Chickens in the XOS, IAPS, and XOS + IAPS groups showed higher serum lysozyme activity and higher IgA-producing cells number of duodenum and ileum than those in the control and AGP group. This is due to XOS and IAPS, which activated the intestinal mucosal immune system and enhanced the intestinal immune barrier function. A better intestinal immune barrier is important for having a healthy growth and so, for animal welfare. However, the authors found in broilers fed on diets supplemented with IAPS or XOS + IAPS a decrease of microbiota richness and an increase of *Ruminococcaceae*, bacteria that produce abundant SCFAs and exert a key role in controlling pathogens. Although this changing, the authors did not find significant differences in the SCFAs concentrations among 5 group.

Yang et al. (2022) investigated if these prebiotics, that alter microbiota's composition and activate metabolic pathways, can also affect meat flavor. They tested if XOS or galacto-oligosaccharides (GOS) can alter this property. GOS consist of 2–10 galactose monomers, synthesized from lactose. They induce beneficial effects on microbiota's growth and activity. Furthermore, they can enhance cell-mediated immune responses. GOS has been repeatedly tested in poultry, where its bifidogenic properties have also been demonstrated (Jahan et al., 2022). Juiciness, flavor, and tenderness, as meat quality characteristics, are mainly determined by the total muscle fat content, intramuscular fat (IMF), and its FA composition. The development of meat flavor and aroma is mainly located in the lipid portion of the meat (Yang et al., 2022). The results were a slight improvement in average body weight, breast muscle percentage, thigh muscle percentage, and abdominal fat percentage, but they were not statistically different. Testing parameters in the chicken blood they found that prebiotic promoted the cholesterol transport, and it was noticed an increase in the activity of lipase and α-amylase in blood, suggesting that prebiotic treatment effectively promoted fat synthesis and starch hydrolysis in chickens, and so a tastier meat. Prebiotic treatments also altered the contents of cecal metabolites related to flavor substances, activating an abnormal gene that involves fatty acid accumulation.

3.3. Arabinoxylans

Arabinoxylans (AX) are non-starch polysaccharides of wheat grains cell wall, but they represent an antinutritional factor for the gastrointestinal tract of broilers, due to their rigid wall structures built of water unextractable arabinoxylans (WU-AX), which enclose highly valuable nutrients together with the viscous high molecular weight water-extractable arabinoxylans (WE-AX). Depending on their physico-chemical properties, like their solubility and extractability, AX can be rapidly or slowly fermented by the intestinal microbial community of the broilers. AX-derived oligosaccharides (AXOS) are smaller oligomers well fermented by intestinal microbiota, inducing beneficial effects on health status of the broilers; they had to be obtained through the hydrolysis of AX, operated by β-1,4-endoxylanases, which are commonly present in chickens' diet. Different studies showed the increase of SCFAs' production, the reduced pH and so the growth of beneficial bacteria in gut, for example *Bifidobacteria*. So, Bautil et al. (2020) set up a study to assess the effects of AXOS addition on AX digestion to see how they act. Four hundred and eighty broilers were divided in two treatments, a control wheat-based diet and the same diet supplemented with AXOS, purified from wheat bran, and consisting for 79 % of arabinose and xylose. A phytase was added in both diets, but no endoxylanase to avoid interference with AXOS. Digesta samples from ileum and caecum were analyzed for AX content, AX digestibility, intestinal viscosity, and microbial AX-degrading enzyme activities at 6 different ages. The authors found that adding AXOS to a wheat-based broiler diet had no impact on feed intake, body weight gain, and feed conversion ratio, because the dose was probably too low to induce better performance. Moreover, ileal viscosity of AXOS-fed broilers was increased compared with the control diet. This result was unexpected with this low dose; the authors explained that probably a part of viscous high molecular weight water-extractable arabinoxylans was accumulated and increased viscosity. AXOS had a stimulating effect on the degradation of dietary fiber by gut microbiota. Providing a highly fermentable dietary fiber source in the starter period trained the metabolic activity of colonizing bacteria in the gut of young broilers, enabling microbiota to start hydrolyzing and fermenting the dietary wheat AX. This kick-starter effect of AXOS on AX digestion is due to a young microbiota, whose composition and metabolic activity can be more easily modified. This study demonstrates that, as previously reported, starter feed and composition are among the most important factors regulating early microbial colonization of the gut.

3.4. Inulin

Inulin is another powerful prebiotic used in animal feed. It consists of a long chain of fructo-oligosaccharides linked by 2,1-glycosidic linkages with a glucose terminal unit and present in many vegetables, fruits, and cereals, for example it can be extracted from chicory roots, onions, bananas, garlic or asparagus, but also from microbial strains of *Aspergillus*, *Fusarium*, *Arthrobacter*, *Aureobasidum*, *Gluconacetobacter*, *Bacillus* and *Saccharomyces*. Dietary supplementation of inulin has been claimed to improve chicken growth performance, carcass yield, activity of the immune system, and important serum biochemical parameters. Inulin added to broilers' diet is fermented in the ceca with a consequentially production of short chain fatty acids, which lower pH in the large intestine and inhibit the growth of pathogenic bacteria, but not in all studies (Xia et al., 2019; Jahan et al., 2022). For example, in a study of Li et al. (2019), the authors investigated the effects of inulin and wheat bran, which is a byproduct of the milling process, rich of insoluble non-starch polysaccharides, such as arabinoxylans (AX) as mentioned before, cellulose, and lignin. They found that single adding of inulin or wheat bran, did not affect BW, BWG and FCR. The combination of wheat bran + inulin group improved BW until day 21. In this study, inulin increased butyrate level, traducing in a major quantity of *Faecalibacterium* and *Anaerostipes*. However, the effect of inulin on butyrate disappeared when inulin supplementation was stopped. Another important result of this study was the reduction of *E. coli* and *Enterobacteriaceae* in wheat bran supplemented diets. The lack of positive effects of inulin might be reconducted to inulin's dose: 2 % inulin in the diet might have been too high for broiler chickens. In fact, in the study of Xia et al. (2019) other results were reported: the authors compared different inclusion dose of inulin (1 %, 2 % and 4 %) to the same diet added with flavomycin. They found that 20 g and 40 g of inulin/kg increased BW gain in the finisher period. The effects of inulin on productive parameters probably vary on the source of inulin used, on the inclusion level, on the composition of the basal diet, on individual animal characteristics, and the state of experimental hygiene conditions, like many other prebiotics. However, in this study, it was found that inulin at all tested levels significantly increased serum concentrations of IgM, improving humoral immunity, like flavomycin group. Finally, inulin improved positive bacteria in gut, like some *Bifidobacterium* strains, especially in the finisher period, when it was found an improvement of BW gain (positive correlation). In fact, *Bifidobacteria* are immunostimulators, competitors of pathogens and producers of energy from volatile fatty acids. The effect of inulin is thought to arise from changes in the expression levels of β-fructofuranosidase genes, encoding invertase-, β-fructosidase-, and inulinase-type enzymes. Furthermore, these bacteria have a fermentative pathway that produces more ATP from glucose or fructose than other bacterial conventional pathways (Xia et al., 2019). In conclusion, it is possible to affirm that inulin acts both as prebiotic and precision biotic, because it affects, not only microbiota composition, but also its pathways.

3.5. Precision biotics

Nowadays, precision biotics are often studied, due to their ability to control nitrogen emissions, with beneficial effects on environment. It is a very important task for researchers to develop new technologies to reduce ammonia emissions in poultry industry (Such et al., 2023). One of these technologies is dietary precision biotics, which can control nitrogen metabolism and protein utilization by the intestinal microbiome, influencing the amount of nitrogen excreted into the environment (Bortoluzzi et al., 2023). Only four studies were found in bibliography about PBs, describing tailored glycans, which are selected for the ability to activate the propionic and butyric acid and bio-synthesis pathways, and to modulate amino acid degradation and amine biosynthesis, reusing nitrogen without excreting it.

The first study divided the experiment in two trials: the first one integrated three different level of PB (0, 250 and 500 g/metric ton) compared with xylanase and a supplementation of both to see possible interactions; the second had only one level of glycans (500 g/mt) compared with a control diet to study the real effect of glycans on broilers' performance. In trial 1, there was not a significant interaction between xylanase and glycans on performance. BW gain and FCR were increased by 250 and 500 g of glycans without the supplementation of xylanase, but the effect of the dose depends on the age of animals, suggesting different response of the microbiota based on age. In trial 2, FI was increased by glycans, which numerically improved BW gain and FCR. In this study, also litter quality, footpad lesions and locomotory problems were evaluated. In this case, glycans and xylanase had an interaction, because litter pH and ammonia concentration in litter were reduced and so, also the footpad lesions and locomotory problems decreased. In trial 2, there was a significant increase of animals without lesions or footpad dermatitis. These necrotic lesions are used as welfare indicators in broilers. They are directly linked to husbandry management and litter conditions, and indirectly to nutrition. Furthermore, ammonia emissions have a negative impact on air quality, eutrophication, and acidification, affecting not only animal welfare as already mentioned, but also human health. In this study, modulating microbial short-chain fatty acids and amino acids metabolic pathways, glycans may have allowed a better use of N in broilers, and so decreased ammonia emissions, improving the litter quality and consequentially, animal welfare (Jacquier et al., 2022).

Blokker et al. (2022) tested the same glycans on broilers undergoing an enteric challenge. Dysbiosis was induced by rapeseed meal and potato protein, which contain antinutritional factors. More stress was added with a 10 dose of coccidiosis vaccines. Dietary supplementation of glycans contained the damage on intestine, increasing goblet cell counts and villi length, they reduced the damage score of liver and restored the IgA levels compared to the control group. It was also observed that challenged broilers supplemented with this precision biotic had an increase of nutrient transporter genes. The authors set a second trial to compare the effect of an animal growth promoter (Avilamycin at 10 ppm) and glycans on chickens raised on reused litter from farms that previously had enteric problems. The results demonstrated that glycans improve growth performance and intestinal health like animal growth promoters. Blokker et al. (2022) concluded that this precision biotic can mitigate the negative impact of an enteric challenge, having a positive effect on the integrity of the intestinal mucosa and on growth performance.

Yan et al. (2023) tested this glycans in a commercial farm. The dietary treatments were two: a control diet and a PB supplemented diet at 0,9 kg/MT. The authors found a significantly increase of BW until day 21 and an improvement of FCR corrected with the final body weight. Furthermore, a significant difference in the cecal microbiome metabolism profile between control and PB supplemented birds emerged from the analysis. The abundance of pathways modulated by glycans involves those associated with amino acid fermentation and other important pathways related to vitamins and carbohydrates. Lastly, the abundance in the cecal microbiome of pathogens (*Escherichia coli* and *Salmonella enteriditis*) was significantly reduced with the supplementation of PB. The results obtained are in accordance with those of Jacquier et al. (2022) and Blokker et al. (2022). Glycans can improve growth performance and reduce environmental impact, increasing the abundance of beneficial pathways and reducing the putrefactive ones.

As the previous studies, Bortoluzzi et al. (2023) observed a significant improvement in BWG and FCR correlated with the final body weight, when broilers were supplemented with 3 % of PB in diet. The influx of glycans into the intestine is one major process that shapes the intestinal microbiome, but the mechanism through which bacteria acquire and utilize glycans is not completely understood. It was observed that the microbiome of heavier birds activated the microbial protein metabolism and shifted the microbiome metabolic functions toward desirable pathways related to nitrogen utilization, resulting in an improvement of growth performance. Moreover, birds fed PB diet reported an increase in the abundance of genes related to propionate production and nitrogen metabolism. This explains why Jacquier et al. (2022), besides improvements in growth performance, observed that the PB improved litter and welfare characteristics, such as footpad score.

4. Conclusions

The analysis of papers published among 2019–2023 shows that prebiotics of different types and origins can influence growth performance and the gut environment, reflecting in better welfare. A healthy gut allows a better use of nutrients and less waste products, mitigating ammonia and other gas emissions (Aruwa et al., 2021).

All the studies reported in this review focus mainly on broilers' performance and gut health, with results depending on many variables. The link between intestinal health and general animal welfare is not deepened, so it would be interesting to investigate further. Animal welfare should not be considered only as the absence of clinical diseases, but also as better reactivity to the stress induced by the environment. Moreover, considering the previous results, the reduction of microbial metabolites and nitrogen excretion with the use of precision biotics may be deepened more in the future, in order to analyze farms' air quality and the reduction of the environmental impact of this sector.

The main limitation of the studies on prebiotics are the results on performance, are variable, depending on many external factors, such as fiber source, level of inclusion, age, physiological status, dietary energy and protein levels, and duration of inclusion, because they act on microbial taxonomy. For this reason, their use is recommended with the implementation of good husbandry management practices, in order to achieve results similar to the inclusion of AGPs. Instead, precision biotics, acting on microbial metabolism pathways, are less influenced by external factors. Furthermore, they have a potential in reducing ammonia emissions. A lower ammonia excretion results in better litter and air quality that allow a better animal welfare and a minor environmental impact. Considering the importance and the increasing interest of people in more environmentally friendly products and in animal health and welfare, precision biotics can be a turning point, even if more research needs to be done.

CRediT authorship contribution statement

Francesca Leone and **Valentina Ferrante**: conceptualization, methodology, writing—original draft and review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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