

## Glimpse of functional feed additives for sustainable broiler production – a review

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**ABSTRACT.** For many years, antibiotics were routinely added to chicken feed to prevent diseases and promote growth. However, growing public health concerns, changing consumer preferences, regulatory reforms, and the global sustainability objectives have led to a significant shift toward antibiotic-free poultry production. A 2020 survey demonstrated that approximately 60% of global poultry producers have transitioned to antibiotic-free practices in response to rising consumer demand. At present, antibiotic-free broiler production is not merely a market trend but a crucial move towards a more responsible industry that prioritises public health, meets consumer expectations, and contributes to global efforts to combat antimicrobial resistance (AMR). The WHO attributes up to 700,000 annual deaths to antimicrobial resistance, with the food industry playing a significant role. In response to ban on certain antibiotics, alternative in-feed additives such as pre-probiotics, enzymes, organic acids (OA), and phytogenic have become popular in poultry production. Research has shown that the use of pre- and probiotics can increase feed intake in broilers by 5–10% and reduce disease by 20–25%. Enzymes, on the other hand, have been shown to improve bird performance and feed efficiency, while mitigating environmental impacts, while OA, when added to poultry feed, inhibit *Salmonella* growth. This review comprehensively examines the mechanisms of action and practical applications of these functional additives, providing valuable insights to support researchers, feed manufacturers, and producers in optimising sustainable, antibiotic-free poultry production systems.

### Introduction

Modern poultry production has made exceptional progress and has become one of the largest and most significant sectors in the global food industry. It integrates systems for breeding, rearing, and processing of various domesticated birds including broiler, ducks, and turkeys, mainly raised for meat and egg production (Abdelli et al., 2021). Modern broiler strains achieve market weight in just 5 weeks through advanced genetic selection and nutritional management (Khan et al., 2022), with projections

indicating further improvements in growth rates and efficiency to meet growing global meat demand by 2050 (Kleyn and Ciacciariello, 2021). In 2017, the EU28 Poultry Meat Export Association reported that the average broiler meat consumption in the United States was estimated at 48 kg/head/year, whereas in Brazil, it was 44.2 kg/head/year (Ayalew et al., 2022). Thus, to maximise production performance, health, and well-being of birds, while minimising environmental pollution, it is essential to identify cost-effective feed additives. Antimicrobial growth promoters (AGP, or antibiotics), were historically

administered in subtherapeutic doses to food animals to enhance growth performance, improve feed conversion efficiency (FCR), and to control enteric pathogens such as those causing necrotic enteritis and coccidiosis (CSCRA, 2016). For instance, Perić et al. (2009) reported that broilers fed a diet supplemented with antibiotics showed a 5.8% increase in body weight (BW). This improvement was attributed to increased appetite, FCR, vitality, and optimised intestinal microflora (Torok et al., 2011). The growth-promoting mechanisms of antibiotics primarily involve modulation of intestinal microbial ecology. As demonstrated by Dibner and Richards (2005), AGP remodel the gut microbial diversity and relative abundance, creating an optimal microbiota profile for nutrient absorption and growth. Specific antibiotic effects on gut microbiota have been well-documented. Fung et al. (2013) showed that 60 ppm salinomycin significantly altered caecal microbiome dynamics in broilers. Similarly, Dumonceaux et al. (2006) reported that application of virginiamycin (100 ppm) as a growth promoter increased *Lactobacillus* abundance in broiler in the duodenal loop and proximal ileum. This indicates that virginiamycin can modify the composition of chicken gut microbiota. However, the widespread use of antibiotics such as bacitracin, salinomycin, penicillin, and virginiamycin (at concentrations ranging from 0.07 to 66 mg/l) in livestock production has raised significant public health concerns due to the emergence of antimicrobial resistance, with potential consequences for both animal and human health (Nhung et al., 2017 and Mehdi et al., 2018). As a result, many countries have implemented regulations to restrict or phase out antibiotics in animal feed. The European Union initiated a ban in 2005 (Dibner and Richards, 2005), followed by South Korean Ministry of Food, Agriculture, Forestry and Fisheries (MAFF) in 2011 (Sampath et al., 2021), The U.S. Food and Drug Administration (FDA) (Zhao et al., 2023) in 2017, while Brazil and China enacted similar regulations in 2018 (Torumkuney et al., 2022) and 2020 (Melaku et al., 2021), respectively. Parallel to these regulatory changes, consumer preferences have markedly shifted toward conventionally produced meat from animals raised without routine antibiotic use, reflecting growing public health concerns and preferences for sustainable agricultural practices. These two scenarios have prompted researchers to explore antibiotic alternatives, specifically natural substances that can enhance poultry growth and feed efficiency while maintaining animal health (Diarra and Malouin, 2014). Consequently, imple-

menting sustainable feeding strategies and identifying effective in-feed additives have become crucial for advancing antibiotic-free broiler production. Feed additives – defined as non-nutritive naturally derived substances incorporated in small quantities into basal diets – play a pivotal role in optimising overall animal performance (Mandey and Sompie, 2021). Natural candidates including probiotics, prebiotics, organic acids (OA), plant extracts and essential oils have gained significant attention in recent decades. These additives are primarily used to improve growth efficiency, feed utilisation, laying capacity, and disease prevention in poultry (Aleli, 2024). However, the precise mechanisms of action for each category, along with their unique bioactive properties, are not fully elucidated. This review systematically explores the underlying mechanisms of these additives and evaluates their impact on production performance. Special emphasis is placed on their potential to transform poultry production systems in the coming decades. A comprehensive summary of common in-feed additives and their efficacy in poultry production is presented in Table 1.

## Potential in-feed additives: mechanism of action and their impact on broiler production

### Prebiotics

Prebiotics are defined as non-digestible feed components that resist host enzymatic digestion but are selectively metabolized by beneficial intestinal microbiota to produce short-chain fatty acids (SCFA), including propionate, acetate and butyrate (Józefiak et al., 2008). These prebiotic components can enhance broiler performance by promoting intestinal health and have emerged as effective alternatives to antibiotic growth promoters (Morales-Lopez et al., 2009). In modern poultry production, the most clinically significant prebiotics are derived from yeast cell walls (*Saccharomyces cerevisiae*) and fermentation byproducts. These include  $\beta$ -glucans, mannan-oligosaccharides (MOS), fructooligosaccharides (FOS), and D-mannose, all of which have demonstrated efficacy in supporting intestinal health (Li and Karboune, 2019). Among these, MOS and FOS prebiotics have emerged as particularly promising alternatives to antimicrobial growth promoters. Recent studies suggest that supplementing MOS to poultry diets can reduce the count of hindgut pathogenic bacteria, particularly during periods of high pathogen exposure

Table 1. Efficacy of Functional feed additives in broilers

Birds (species)	Dose	Additive	Main findings	Reference
Turkey	0.4%	Mannan-oligosaccharides (MOS)	Lowering ammonia (NH <sub>3</sub> ) concentration	Juskiewicz et al. (2003)
Male Cobb 500 broilers	0.2% MOS + 1.25% Alcell lignin	Mannose and mannoproteins	Significant increases in villus height and goblet cell numbers. Reduction in <i>Escherichia coli</i> load may help control cellulitis	Baurhoo et al. (2007)
25-d-old Ross 308 growing broiler chickens	0.4% Gly, 0.8% Gly, and 1.6% Gly	Glycine	Improved feed efficiency, meat quality, liver health, and intestinal morphology	Kwon et al. (2024)
One-day-old broilers	<i>in ovo</i> + in-water DN (Dinovo®), extract of beta-glucans) and BI (Bi2 tos, trans-galactooligosaccharides)	Prebiotics	Increased FI and FCR. Enhanced intestinal health. Developed intestinal microflora.	Bednarczyk (2016)
One-day-old broilers	2 levels of β-glucan (0 or 1 g/kg) and <i>Bacillus subtilis</i> (0 or 108 CFU/kg)	β-glucan	Improved body weight gain (BWG) in broilers. Relative spleen weight increased with improved BWG. β-glucan at 1 g/kg reduced the b (yellowness) value of breast meat	Zhang et al. (2012)
One-day-old broilers	0, 1, and 2 g/kg feed	MOS	Improved growth performance and antibody titres against IBD in broilers fed antibiotic-free diets	Rehman et al. (2020)
One-day-old male Ross 308 broiler chickens	0.025% eubiotics of microbial muramidase + 0.1% precision glycan	Eubiotics of microbial muramidase (MR) and precision glycan	Increased growth performance, total serum carotenoid level and altered gut microbiota composition	Cho et al. (2024)
One-day-old Ross 308 broiler chickens	0%, 0.05%, 0.1%, and 0.2%	Multi-probiotics ( <i>Bacillus subtilis</i> and <i>Clostridium butyricum</i> )	Improved ADG by increasing ADFI and modulating the caecal microbe	Zhang et al. (2023)
One-day-old Ross 308 broiler chickens	200 g/t probiotic mixture	Multi strain probiotics ( <i>Bacillus coagulans</i> , <i>B. licheniformis</i> , <i>B. subtilis</i> , <i>Clostridium butyricum</i> )	Beneficial effects on growth performance, drip loss percentage and faecal <i>Lactobacillus</i> counts	Balamuralikrishnan et al. (2017)
Broilers and turkeys	0, 300, or 600 g/kg wheat-DDGS 2 levels of XAP (0 or 250 mg/kg diet)	Protease to wheat-DDGS with soluble-based diet	Supplemental XAP increased metabolizable energy in wheat-DDGS by up to 203 kcal/kg DM	Adebiji and Olukosi (2015)
Two-week-old broilers (Ross-308)	Crude protein levels (CP, 17, 19 and 21%) and two levels of exogenous protease (0 and 30 000 IU/kg)	Microbial protease enzyme and dietary crude protein levels	Improved growth performance and nutrient digestibility	
One-day-old Ross broiler chickens	PAN8906 with 500 FTU/kg phytase with or without 1600 BXU/kg xylanase).	Sorghum-based diets with xylanase	Xylanase supplementation did not improve the performance and nutrient digestibility	O'Neil et al. (2017)
One-day-old male Ross 308 broiler chickens	Two levels of protease (0 or 0.02%) and EO (0 or 0.03%).	Protease	Improved growth performance, increased ileal <i>Lactobacillus</i> populations, and reduced ileal <i>E. coli</i> populations	Park and Kim (2018)
One-day-old male Ross 308 broiler chickens	0, 1, 875, 3, 750, and 5,625 XU/kg xylanase	Xylanase	Decreased ileal digesta viscosity and improved growth performance and apparent ileal digestibility of nutrients	Liu and Kim (2017)
One-day-old male Ross 308 broiler chickens	0.1% protease	Low CP diet with protease	Improved AA digestibility, consequently improving BWG and FCR	Mohammadigheisar and Kim, 2018

MOS – mannan-oligosaccharides; NH<sub>3</sub> – ammonia; Gly – glycine; β-glucan – beta glucan; FI – feed intake; FCR – feed conversion ratio; BWG – body weight gain; ADG – average daily gain; ADFI – average daily feed intake; AA – amino acid; CP – crude protein; DDGS – distillers dried grains with soluble; XAP – xylanase, amylase, and protease; DM – dry matter; EO – essential oil; FTU – phytase unit (one FTU is the amount of phytase that releases one micromole of inorganic phosphate per min); IBD – infections/bursal disease

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Table 1. Efficacy of Functional feed additives in broilers (continued)

Birds (species)	Dose	Additive	Main findings	Reference
One-day Ross broiler chickens	1500 FTU/kg	Phytase	No positive effect on growth performance, organ weight and tibia ash in broilers	Kumar and Kim (2022)
One-day-old broiler chicks (Hubbard Classic)	0.5%	Citric acid	Improved broiler growth, bone ash deposition, and immune status against enteric pathogens and infectious diseases	Chowdhury et al. (2009)
One-day-old unsexed Ross 308 broilers	0 or 1 g/kg blend of organic acids	Combination of sodium butyrate, citric acid, phosphoric acid, acetic acid, propionic acid, formic acid, and lactic acid	Improved growth performance	Sabour et al. (2019)
Cobb straight run commercial broilers	2 and 3% butyric acid, 2 and 3% fumaric acid, 2 and 3% lactic acid	Combination of butyric acid, fumaric acid and fumaric acid	Improved small intestine histology, nutrient absorption and broiler growth	Adil et al. (2010)
68-week-old Hy-Line	0.5, 1.0- and 1.5-ml l <sup>-1</sup>	Formic acid	Improves egg production, weight, shell quality, grading, and boosts immunity in older layers during hot weather	Abbas et al. (2013)
One-day-old e Ross 308 broiler chickens	0, 0.25, 0.50 or 1.00 g sodium butyrate/kg	Sodium butyrate	Improved growth performance under stress condition, regulated the immune response, minimizing tissue damage	Zhang et al. (2011)

MOS – mannan-oligosaccharides; NH<sub>3</sub> – ammonia; Gly – glycine; β-glucan – beta glucan; FI – feed intake; FCR – feed conversion ratio; BWG – body weight gain; ADG – average daily gain; ADFI – average daily feed intake; AA – amino acid; CP – crude protein; DDGS – distillers dried grains with soluble; XAP – xylanase, amylase, and protease; DM – dry matter; EO – essential oil; FTU – phytase unit (one FTU is the amount of phytase that releases one micromole of inorganic phosphate per min)

(Castillo et al., 2008). Similarly, Juśkiewicz et al. (2003) found a decrease in ammonia emissions in turkeys with the inclusion of MOS supplement. Literature also documents that dietary MOS can significantly lower the number of pathogens. Some studies have proved that MOS supplementation exerts a considerable effect on gut histological structure in broilers. For instance, Baurhoo et al. (2007) reported that chicks fed a diet supplemented with mannose and mannoproteins showed a significant increase in the intestinal villus cells. More recently, Kwon et al. (2024) demonstrated that dietary mannan-based supplements have increased the villus height-to-crypt depth ratio in young broilers, indicating improved nutrient absorption capacity. Nochta et al. (2010) also found a remarkable improvement in apparent nutrient digestibility of broilers with response to mannan supplementation, while Bednarczyk et al. (2016) demonstrated that 0.2% mannanoligosaccharide inclusion in the chicken diet conferred intestinal health benefits exceeding antibiotic effects. These advantages are mediated through three primary mechanisms: competitive exclusion of pathogenic bacteria, improved intestinal morphology (increased villus height and goblet cell density), and an increased colonisation by beneficial bacteria. Zhang et al. (2012) demonstrated that  $\beta$ -glucan supplementation significantly reduced the relative abdominal fat weight of broilers by 16.0% ( $P < 0.05$ ). Additionally, Rehman et al. (2020) reported improved growth performance and antibody titre against infectious bursal disease in broilers following dietary prebiotic inclusion. The unique chemical stability of prebiotics allows them to resist degradation by enzymes in the digestive tract, reaching the intestine where they selectively stimulate beneficial microbial populations. These microbes metabolise prebiotics to generate valuable compounds including short-chain fatty acids, essential vitamins, and natural antimicrobial substances that support gut health while inhibiting pathogenic microorganisms. Moreover, the fermentation products generated by beneficial gut microbiota significantly strengthen intestinal epithelial barrier function, leading to improved nutrient absorption and overall growth performance of animals. Prebiotic-mediated modulation of the gut microbiome is closely associated with immune system regulation. Oligosaccharides are known for their immunomodulatory properties within the gastrointestinal tract, including promoting pathogenic bacterial clearance, activating T cell-dependent immune responses, and suppressing proinflammatory cytokine activity (Bonos et al., 2010). Through

competitive prevention of pathogen colonisation, prebiotics effectively reduce the concentration of pathogen-associated molecular patterns (PAMP) produced by harmful microorganisms. These molecules are detected by pattern recognition receptors (PRR), such as toll-like receptors (TLR) and NOD-like receptors (NLR) expressed on the surface of sentinel cells (Amarante-Mendes et al., 2018). Upon binding to PAMP, these PRR activate sentinel cells, including macrophages, epithelial cells, dendritic cells, and mast cells, triggering cytokine release and initiating innate immune responses. These signals are subsequently detected by immune cell receptors, thereby contributing to the modulation of the host's immune system. Dietary supplementation with prebiotics, particularly MOS and FOS, has been shown to improve broiler performance through their effects on gut health, pathogen reduction, and immune modulation. These combined effects result in improved growth, nutrient absorption, and overall animal health. The increased production of short-chain fatty acids (SCFA) like acetate, propionate, and butyrate during the fermentation of prebiotics supports gut health by reducing harmful bacteria while stimulating the growth of beneficial gut microbiota. This leads to healthier broilers with improved feed conversion rates, reduced pathogen load, and better overall performance, contributing to higher-quality poultry products. Additionally, studies suggest that prebiotics like  $\beta$ -glucan may contribute to improved meat quality by reducing abdominal fat deposition in broilers. The beneficial effects of prebiotics extend beyond gut health, as they also enhance immune function, leading to more robust disease resistance and potentially safer meat products with lower microbial contamination. Based on existing scientific evidence, optimal broiler productivity may be supported through dietary inclusion of 0.1 to 0.5% MOS and FOS, along with 0.1 to 0.25%  $\beta$ -glucan in total feed.

### Probiotics

Probiotics are live beneficial microorganisms that promote host health by maintaining intestinal microbial balance and physiological functions. These microorganisms are resistant to gastric acid, bile salts, and digestive enzymes, which allows them to colonize the gastrointestinal tract and effectively compete with pathogenic bacteria. Their mechanisms of action include competitive exclusion, immune modulation, and metabolic regulation, leading to improved animal health and productivity (Sampath et al., 2022). In poultry production, probiotics can also increase

feed efficiency, regulate apoptosis, increase energy digestibility, and improve the breakdown of non-starch polysaccharides (NSP) while positively influencing caecal and litter microbiota. Different types of probiotics have been shown to inhibit pathogenic bacteria both *in vitro* and *in vivo* through a variety of mechanisms. The most widely used probiotics in poultry include Gram-positive bacteria such as *Bacillus*, *Lactobacillus*, and *Pediococcus*, along with fungi like *Aspergillus* and *Saccharomyces* (Zomiti et al., 2020). Research indicates that probiotic efficacy varies by host species, with *Saccharomyces cerevisiae* and *Aspergillus oryzae* being particularly effective in ruminants, while *Lactobacillus* strains showing more beneficial outcomes in poultry. Probiotics maintain intestinal homeostasis through competitive inhibition of pathogens while modulating microbial metabolism by enhancing digestive enzyme activity and reducing bacterial enzyme activity and ammonia production (Markowiak and Śliżewska, 2018; Aziz Mousavi et al., 2018). A study by Van Immerseel et al. (2006) demonstrated that dietary *Lactobacillus* improved the egg quality and reduced *Salmonella* contamination in chickens. Similarly, Jungersen et al. (2014) reported that specific *Bifidobacterium* strains (*Bifidobacterium animalis*, *B. thermophilum*, and *B. longum*) effectively reduced the coccidiosis in broiler infected with *Eimeria tenella*. Recent work by Cho et al. (2024) revealed that broilers receiving microbial muramidase (MR) and precision glycan (PG) as dietary eubiotics exhibited significant improvements in body weight gain, feed intake, serum carotenoid levels, and ileal microbial diversity compared to control groups. Zhang et al. (2023) reported a linear increase in average daily gain, daily feed intake, and a reduction in *Clostridium perfringens* counts in broilers supplemented with increasing levels of multi-probiotics consisting of *Bacillus subtilis* and *Clostridium butyricum*. Biswas et al. (2023) further highlighted the positive effects of probiotics (*Bacillus subtilis*  $7.0 \times 10^7$  CFU/g, *Bacillus licheniformis*  $4.1 \times 10^7$  CFU/g) on broiler performance. Although *Enterococcus faecium* has been shown to improve feed conversion ratio (FCR) and modulate caecal microflora in broilers (Lan et al., 2017), safety concerns remain as some strains are associated with antibiotic resistance transfer and urogenital tract infections and endocarditis (Garsin et al., 2014). Therefore, it is essential to ensure that strains pose no health risks to animals before application in feeds. Probiotics exert nutritional effects by increasing fibre digestion and enzymatic activity, thereby

improving nutrient utilisation efficiency in poultry. Research by Wang et al. (2018) demonstrated that supplementing broilers with *Bacillus subtilis* was particularly effective under heat stress conditions through microbiota-mediated immunomodulation. However, contrasting results from Balamuralikrishnan et al. (2017) showed no significant impact on feed intake or FCR with multi-strain probiotics, suggesting strain-specific and condition-dependent effects. Probiotics influence the intestinal microbiota composition of poultry, with their effectiveness largely dependent on the presence of undesirable microbial populations that may impair growth. One of the primary mechanisms involves acidification of the gut environment through production of volatile fatty acids and organic acids during their breakdown. The lower pH creates unfavourable conditions for pathogenic colonisation in the digestive tract, effectively preventing harmful microbes through competitive inhibition. However, in cases where the gut microbiota is already balanced and free of growth-limiting pathogens, probiotic supplementation may show limited effects. Optimal and safest dosing of probiotics in poultry diets varies depending on the specific strain and the desired outcomes, whether for growth performance, pathogen control, or immune modulation. Based on current research, the following probiotic dosages are recommended for optimal efficacy in broiler production: *Bacillus subtilis* ( $1.0 \times 10^7$  to  $1.0 \times 10^9$  CFU/g), *Lactobacillus* ( $1.0 \times 10^6$  to  $10^8$  CFU/g), *Saccharomyces cerevisiae* ( $1 \times 10^6$  to  $1 \times 10^8$  CFU/g), and *Enterococcus faecium* ( $1.0 \times 10^7$  to  $10^9$  CFU/g). These concentrations have demonstrated safety and efficacy in improving broiler growth rates, egg quality, and resistance to pathogens when administered under appropriate conditions.

## Enzymes

As non-traditional feed ingredients gain popularity in poultry production, the use of exogenous enzymes has become increasingly important. To reduce the cost of ration formulation, alternative feed ingredients and lower-protein diets have recently been introduced. Particularly, soybean meal (SBM), is widely applied as a primary protein source, while yellow maize is the predominant energy source in animal feed. Enzymes are catalysts produced by cells to accelerate specific chemical reactions, including those involved in digestion (Ayalew et al., 2022). They can be derived from both plant and microbial sources, with significant research attention directed toward phytase production from

filamentous fungi, including *Aspergillus*, *Myceliophthora*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichoderma* (Jatuwong et al., 2020). The poultry industry currently utilises three main classes of feed enzymes: phytases, carbohydrases (non-starch polysaccharides, NSP), and proteases. Market trends over the past five years have promoted phytases as the dominant enzyme category, followed by xylanases, with cellulases (glucanases) representing a smaller market segment. Phytases provide crucial economic and environmental benefits by reducing reliance on costly inorganic phosphorus supplements and minimising phosphorus pollution from manure. This is particularly significant as 60–75% of phosphorus in feed grains exists as phytate, an organic form indigestible by monogastric animals (Steiner et al., 2007). In addition to phosphorus liberation, phytases mitigate the antinutritional effects of phytic acid while increasing mineral and protein bioavailability, thereby improving overall bird health. The recent surge in dietary energy costs has prompted nutritionists to reconsider the value of NSP enzymes as potential cost-saving tools in poultry feed formulations. Unlike wheat-based diets, where enzyme targets the reduction of intestinal viscosity from soluble fibres, maize-soybean meal diets require a different approach due to their low soluble fibre content. In these formulations, NSP enzymes are believed to primarily target insoluble fibre components to disrupt cell wall structures and improve nutrient accessibility. Research indicates that high levels of NSP in poultry diets lead to intestinal hypertrophy, increasing gut mass and associated maintenance costs (Morgan et al., 2022). These modifications to the digestive tract lower digestive efficiency and increase the nutrient requirements for gut maintenance, consequently diverting nutrients away from growth processes. In addition to phytase and NSP enzymes, proteases represent another important class of feed enzymes. These proteolytic enzymes, which hydrolyse peptide bonds in proteins, are widely distributed in plants, animals, and microorganisms. Previous study by Bekhit et al. (2014) have reported that exogenous proteases such as collagenase from *Clostridium histolyticum*, aspartic protease from *A. oryzae*, thermophile protease from a *Bacillus* strain, and caldolysin from a *Thermus* strain are commercially used to improve meat tenderness. In addition, Adebisi and Olukosi (2015) demonstrated that the addition of 300 or 600 g/kg of a carbohydrase and protease mixture to a diet based on wheat distillers' dried grains with solubles (DDGS) increased metabolizable energy in broilers

and turkeys. More recent work by Jabbar et al. (2021) found that including 1% prilled palm fat combined with lyso-lecithin to broiler diets positively affected nutrient digestibility, body weight gain, and FCR. Adding enzymes to broiler diets offers nutritional, economical, and environmental benefits (Dokovic et al., 2013). Extensive research has shown that supplementing broiler diets with combinations of xylanase, amylase, and protease improves nutrient digestibility and growth performance. For instance, Cowieson and Ravindran (2008) observed that broilers fed a diet supplemented with a combination of xylanase, amylase, and protease (500 mg/t) increased energy availability by 3–5%. Similarly, O'Neil et al. (2017) showed improved weight gain, feed intake, crude protein digestibility, and nitrogen and fibre absorption in broilers fed xylanase-supplemented sorghum-based diets. These positive effects may result from the synergistic action of enzymes on various chemical bonds in indigestible feed ingredients, thereby increasing the energy available for growth. Research by Park et al. (2018) demonstrated that incremental dietary protease supplementation (0–0.09%) in broiler diets linearly increased their BW, ADG while reducing FCR. The latter authors also reported linear improvements in the digestibility of DM, CP, DE, and amino acids, as well as the upregulation of growth-related genes such as *IGF1*, *IGF2*, *GH*, and *LEP* in the liver, and *MYOD1* and *MYOG* in breast muscles. These dose-dependent improvements likely result from enhanced protein hydrolysis and amino acid availability due to protease action, which optimises energy metabolism and stimulates expression of growth-related genes, ultimately leading to better nutrient utilisation and growth performance in poultry. Similarly, Liu and Kim (2017) reported increased BW and reduced FCR and ileal digesta viscosity in broilers fed diets supplemented with xylanase. The researchers also observed significant morphological improvements in the small intestine, including increased villus height and villus height-to-crypt depth ratios in the duodenum, jejunum, and ileum. Xylanase specifically targets and breaks down xylans, a type of NSP prevalent in grains like wheat and barley. These NSP are indigestible for poultry and tend to increase digesta viscosity, thereby impairing nutrient absorption. By breaking down these complex polysaccharides, xylanase reduces digesta viscosity, leading to improved nutrient diffusion and absorption in the intestines – mechanisms that collectively improve BW and FCR. Additionally, increased villus height and villus-to-crypt ratio enhance nutrient

absorption and create a less favourable environment for pathogenic bacteria. This adaptation likely occurs in response to reduced digesta viscosity and improved gut health, facilitating more efficient nutrient uptake. Mohammadigheisar and Kim (2018) observed that chicks fed a low CP diet supplemented with protease showed higher BWG and DM digestibility. However, they also noted a negative impact on the digestibility of total essential amino acids (TEAA) and total non-essential amino acids. The positive effect of phytase supplementation on broiler performance was also reported by Dang et al. (2022) and Sampath et al. (2023). However, Kumar and Kim (2022) showed that high phytase inclusion levels (1,500 FTU/kg) produced limited improvements in ADFI and FCR, while adversely affecting meat quality. These outcomes could be attributed to excessive phytase levels disrupting nutrient balance, altering amino acid and mineral availability, and inducing oxidative stress. These metabolic disturbances can compromise meat quality parameters despite potential modest gains in growth performance parameters (ADFI and FCR). Based on current evidence, optimal enzyme supplementation levels for broiler diets should be carefully calibrated within the following ranges: phytase at 500–1 500 FTU/kg (fungal phytase units per kilogram of feed), xylanase at 500–1 000 mg/t of feed, and protease at 300–600 g/kg. These dosages are considered safe and effective for promoting growth and feed efficiency. Excessive phytase supplementation, exceeding 1 500 FTU/kg, may induce nutrient imbalances and potentially compromise meat quality (Kumar and Kim (2022)). Therefore, careful optimisation of enzyme dosages is necessary to maximise benefits while avoiding adverse outcomes.

### Organic acids

Currently, a wide variety of ingredients and by-products are applied in the poultry industry to reduce costs and improve performance. OA, a group of carboxylic acids including fatty acids with the R-COOH chemical structure (Abbas et al., 2022) has been widely utilised in poultry production as feed additives and/or in drinking water to minimise the effects of feedborne pathogens like *Salmonella* spp. Dietary acids used in poultry can be classified into two categories: 1) inorganic acids (IOA) and 2) OA. Compared to IOA, OA are more effective in improving gastric proteolysis and amino acid and mineral digestibility (Nguyen et al., 2020). Acids such as butyrate, propionate, and acetate are naturally produced in the anaerobic environment of the

gastrointestinal tract (GIT). OA typically consists of saturated monocarboxylic acids or their derivatives (Ricke 2003). Previous studies have demonstrated the positive effects of OA in broiler rearing. For instance, Baghban-Kanani et al. (2023) reported that dietary inclusion of OA improved feed utilisation and reduced pathogenic microbial loads in broilers. Similarly, Chowdhury et al. (2009) observed that adding 0.5% citric acid to broiler diets enhanced growth rate, feed intake, carcass yield, and bone ash levels. Adhikari et al. (2020) also reported that 0.9% dietary OA has improved the growth rate in broilers infected with *Salmonella typhimurium*. Moreover, Sabour et al. (2019) observed an increased growth rate in broilers receiving a blended OA formulation containing sodium butyrate, citric acid, phosphoric acid, acetic acid, propionic acid, formic acid, and lactic acid. The improved performance of broilers with OA supplementation can be attributed to increased digestibility of energy and protein in the feed, as well as a reduced abundance of microbial pathogens. Meanwhile, the FCR likely results from lower feed intake, combined with better nutrient utilisation, leading to higher weight gains in broilers. Another reason could be the proliferation of pathogenic microbes or bacteria in the gastrointestinal tract, which can damage villus structures and trigger excessive epithelial cell proliferation, leading to thickened intestinal membranes. This pathological thickening may impair nutrient absorption capacity and consequently affect growth performance. Several studies have demonstrated the positive effects of organic acids (OA) on nutrient digestibility in broilers (Rodjan et al., 2017; Sureshkumar et al., 2021). Chowdhury et al. (2009) noted increased phosphorous utilisation in broilers supplemented with citric acid, while Garcia et al. (2007) observed improved growth performance and apparent ileal digestibility in birds receiving 5 000–10 000 ppm formic acid (FA). However, Hernández et al. (2006) found minimal effects on nutrient digestibility at lower FA inclusion levels (5–10 g/kg), potentially due to pH-mediated modulation of specific microbial populations affecting nutrient digestion and absorption. Research by Rodríguez-Lecompte et al. (2012) revealed that OA-supplemented diets significantly increased villus height and surface area in the duodenum, jejunum and ileum of chicks. Complementary findings by Khan and Iqbal (2016) showed that chicks fed diets containing OA blends had higher levels of beneficial bacteria, such as *Lactobacilli*, while reduced populations of harmful bacteria, like coliforms and *Clostridia*, in the ileum. The increased presence of



*Lactobacilli* in OA-supplemented broilers may be attributed to their lower susceptibility to pH fluctuations, whereas coliforms are more sensitive to pH reductions in the gastrointestinal tract. Adil et al. (2010) observed that dietary supplementation of broilers with 3% butyric acid, 3% fumaric acid and 2% fumaric acid resulted in significantly increased villus height in the duodenum, jejunum, and ileum, respectively. The morphological improvement in individual segments of the small intestine likely reflects the role of the intestinal epithelium as a protective barrier against pathogens and toxic substances in the lumen. In modern poultry production, broilers are often characterised by less robust immune systems. The rapid growth and efficient feed utilisation frequently result in compromised immune function (Ghazvinian et al., 2018). The accelerated growth outpaces immune system development, increasing disease susceptibility. The inclusion of OA in their diet can regulate pathogenic bacterial populations, potentially enhancing immune resilience. Abbas et al. (2013) observed significantly higher Newcastle disease antibody titres in OA-supplemented broilers, while Zhang et al. (2011) observed that sodium butyrate reduced oxidative stress through increased antioxidant activity, potentially via NF- $\kappa$ B pathway inhibition. However, contrasting findings by Levy et al. (2015) showed no intestinal health improvements with OA supplementation, highlighting the influence of variables like microbial challenges, bird genetics, acid type/dosage, and feed composition. Despite these inconsistencies, OAs are widely regarded as effective additives for improving broiler growth performance, nutrient digestibility, and gut health. Safe and effective supplementation typically ranges between 0.5 and 3% depending on the specific acid used. Combining different OAs can exert synergistic effects, thus careful consideration of dosages and combinations is essential to optimize outcomes while avoiding adverse effects on meat quality or intestinal health.

### Phytogenic

Phytogenic feed additives (PFA) are biologically active compounds increasingly used in poultry production due to their beneficial effects and cost-efficiency. These plant-derived additives are broadly categorized as herbs/spices or based on extraction methods, as essential oils (EO) and oleoresins (Abdelli et al., 2021). Their non-toxic, economical, and environmentally sustainable properties make them particularly attractive for modern poultry production (Madhupriya et al., 2018). Various plants,

including basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), alfalfa (*Medicago sativa*), and chlorella (*Chlorella vulgaris*), are generally recognised as safe (GRAS) for animal feed applications (Martinez-Mayorga et al., 2013). Research has consistently demonstrated the growth-promoting effects of specific PFA. Gurbuz and Ismael (2016) reported that 1.5% peppermint (*Mentha piperita*) supplementation significantly enhanced broiler growth performance, while Al-Kassie (2010) observed improved body weights following supplementation of this PFA. In addition, Abbas (2010) observed that broilers fed diets supplemented with 3 g/kg basil achieved the best FCR value. Recent advancements in extraction techniques and the identification of active ingredients have accelerated the adoption of phyto-genic extracts as alternatives to antibiotics in animal nutrition. Herbal products are typically incorporated into animal feed in dried and crushed forms or as crude extracts containing active herbal ingredients (Wang et al., 2024). Shamma et al. (2019) study showed that broilers fed a diet supplemented with 0.3 ml/l thyme oil had significantly increased body weight, body weight gain and improved FCR, along with increased antibody titre against infectious bursal disease (IBD). These positive findings could be attributed to the phenolic components in thyme, which contribute to improved growth and nutrient digestibility in broilers. Several studies have documented the beneficial effects of sage supplementation in poultry production. Levkut et al. (2010) demonstrated improvements in growth performance and feed conversion ratio with sage inclusion. More recently, Bahadoran et al. (2023) found that 0.2% dietary sage modulated pulmonary hypertensive responses, enhanced antioxidant status through increased enzymatic activity, and improved intestinal morphology, expanding absorptive surface area in broilers. Similarly, He et al. (2021) observed significant linear increases in body weight and average daily feed intake in birds supplemented with 75 g/kg alfalfa meal. The inclusion of alfalfa meal also resulted in significantly lower pH in the duodenum, caecum, and breast muscle. Furthermore, Vlaicu et al. (2022) reported that combined supplementation of basil, thyme and sage had a beneficial effect on broiler meat quality parameters. Secondary plant metabolites (alkaloids, glycosides, flavonoids, phenolic acids, saponins, tannins, terpenes, anthraquinones, and steroids) and bioactive compounds exert notable physiological and pharmacological effects in animals (Roy et al., 2022). Research by

Begum et al. (2014) demonstrated a dose-dependent response to *Platycodi radix* extract (0–0.15% inclusion), with linear improvements in BWG, energy digestibility, bone strength, and insulin-like growth factor levels in broilers. Subsequent work by Li et al. (2015) confirmed that phytoncide supplementation improved growth efficiency while reducing noxious gas emissions from poultry production. Additionally, dietary supplementation with 0%, 0.2%, 0.4%, and 0.6% quercetin has been shown to improve body weight gain and feed intake in 35-day-old broilers (Dang et al., 2022). This positive outcome could be attributed to several factors, including the botanical source, concentration, supplementation duration, feed composition, experimental conditions, and the age and health status of the animals. In addition to herb extracts, Essential oils (EO) have emerged as particularly promising phyto-genic additives in poultry nutrition. Recent research has demonstrated the efficacy of various EO, including cinnamaldehyde (Paraskeuas et al., 2017), *Citrullus lanatus* (Marume et al., 2020), peppermint oil (Abdel-Wareth et al., 2020), and tea tree (Puvača et al., 2020). Liu et al. (2018) specifically investigated carvacrol essential oil and confirmed its positive effects on intestinal barrier function in broilers. Plant extracts have also shown significant potential. Park and Kim (2020) reported improved broiler growth performance with the supplementation of *Achyranthes japonica* extract (AJE), while Abd El-Hack et al. (2018) and Shokrollahi and Sharifi (2018) observed enhanced growth in both broilers and laying hens supplemented with black cumin (*Nigella sativa*) seeds. However, contrasting results were reported by Karadağoğlu et al. (2019), who found no significant difference in growth performance of Japanese quails fed diets contain black cumin. Hussein et al. (2020) found that a combination of peppermint, chamomile, and prebiotic yeast cell wall was as effective as salinomycin in maintaining weight gain and FCR in coccidiosis-challenged broilers. The effectiveness of yucca-derived saponins on growth performance depends on the broiler's health status. Su et al. (2016) reported that yucca saponins promoted growth in healthy broilers, while Oelshlager et al. (2019) found no significant effect during active coccidial infection, suggesting reduced bio-efficacy during immune stress. This highlights the importance of evaluating digestibility, as it directly influences feed efficiency in poultry production. Improving digestibility not only enhances feed efficiency but also reduces undigested feed residues in the gut, thereby preventing intestinal disorders. Abdel-Wareth et al. (2020)

demonstrated that dietary supplementation with 0, 74, 148, 222, and 296 mg/kg of peppermint oil linearly increased the digestibility of CP, ether extract (EE), and phosphorus in 44-week-old laying hens (Abdel-Wareth et al., 2020). Complementary findings by Dang et al. (2021) showed that YGF251 supplementation – a herbal pharmaceutical extract stimulating insulin-like growth factor-1 secretion – significantly enhanced protein utilisation and reduced ammonia excretion in laying hens, highlighting the potential of plant-derived compounds to improve nitrogen metabolism. Phyto-genic additives also exhibit protective effects against mycotoxins and inflammatory responses. Rashidi et al. (2020) documented that dietary licorice extract mitigated liver damage in broilers caused by aflatoxin B1, as evidenced by lower alkaline phosphatase, aspartate aminotransferase, and alanine transaminase activity, and decreased malondialdehyde (MDA) concentration in breast meat. Moreover, Pirgozliev et al. (2019) observed that a phyto-genic blend containing carvacrol, cinnamaldehyde, and capsicum oleoresin downregulated pro-inflammatory cytokines (interferon- $\gamma$  and IL-6) in broilers under non-challenged conditions, suggesting immunomodulatory properties. The beneficial effects of PFA on broiler and layer performance are primarily attributed to their digestion-stimulating properties, which improve nutrient absorption of key elements, including selenium, B-complex vitamins, and  $\beta$ -carotene. Although some studies reported a reduction in populations of beneficial bacteria like *Lactobacillus* following plant extract supplementation, others demonstrated an increased abundance of beneficial bacterial orders like *Clostridiales* or *Lactobacillales* in broilers supplemented EO. For example, Perricone et al. (2020) found that a plant extract from green tea and pomegranate increased the count of lactic acid bacteria, which play crucial roles in maintaining intestinal homeostasis and enhancing immune function. Compared to antibiotics, phyto-genic products offer distinct advantages, including cost-effectiveness and a lower risk of resistance development, making them sustainable alternatives for improving various production parameters. These natural compounds have proven effective in improving growth, performance, health status, reproductive efficiency, and environmental sustainability through reduced emissions in monogastric animals. However, evaluating PFA remains challenging due to the wide variability in extracts and formulations. Standardising evaluation systems is essential to enable conclusive assessments and further increase poultry production efficiency.

## Conclusions

Effective nutrition is key to sustainable poultry production, enhancing feed utilisation and reducing environmental impact. By optimising feed conversion efficiency, poultry operations can improve their financial viability while lowering resource demand and carbon footprint. However, the environmental effects of grain and soybean production, coupled with long transport chains, require careful consideration. Ingredients should be assessed based on their specific production methods, such as precision farming and conservation tillage, to minimise environmental degradation. Future research should focus on refining these practices and exploring novel feed ingredients to further address sustainability challenges. Aligning these strategies with global sustainability goals will strengthen both environmental and economic stability in poultry farming.

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## Conflict of interest

The Authors declare that there is no conflict of interest.

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