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Phenomenological and mechanistic insights into potential dietary nucleotide – probiotic synergies in layer chickens: A review

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A B S T R A C T

Despite their growing popularity as alternatives to antibiotic growth promoters (AGPs), the individual effects of nucleotides and probiotics on poultry gut functionality remain poorly understood. In addition, inconsistent outcomes are quite common in studies where these two additives have been used separately to modify gut function and related parameters in birds. These inconsistencies, which have limited the potential of probiotics and nucleotides as AGP replacements, stem from various factors and need to be addressed. Combining probiotics and nucleotides could potentially enhance their effectiveness and lead to more consistent outcomes in layer chickens. Since their mechanisms of action complement each other, some level of synergy is expected when used together. Both additives have been shown to support gut health, boost immune function, and improve performance in chickens when used individually. However, no studies have investigated the possible synergistic effects of nucleotides and probiotics in poultry. This review makes the case for combined use of probiotics and nucleotides in layer chickens by providing phenomenological and mechanistic insights into hypothetical synergistic effects. This paper highlights the need for AGP alternatives and reviews studies on the effects and mechanisms of probiotics and nucleotides in layer chickens when used individually. We then propose potential mechanisms for their synergistic effects on gut health, performance, and egg quality based on logical deductions from observed biological responses. These proposed mechanisms are hypothetical and require experimental validation. Finally, the review explores how this synergy could lead to more consistent outcomes and enhance the feasibility of AGP-free egg production.

Introduction

The chicken's gastrointestinal tract plays key roles in digestion, absorption, metabolism, immunity, and endocrinology (Svihus, 2014). Disruptions to its function can negatively impact bird health, welfare, production efficiency, product quality, and environmental health. Traditionally, antibiotic growth promoters (AGPs) have been widely used to counteract stress-induced gut disruptions. However, their indiscriminate use is recognized as a major driver of antimicrobial resistance in pathogens affecting both humans and animals (Phillips et al., 2004). Additionally, the presence of AGP residues in poultry products can negatively affect consumer health. As a result, consumers often perceive antibiotic-free poultry as superior to conventionally raised poultry (Cervantes, 2015), making it more socially acceptable. The ban or restriction of antimicrobial growth promoters, along with efforts to reduce therapeutic antibiotic use in some countries, has led to significant issues of gut dysbiosis in farmed animals, including chickens (Onrust et al., 2015). However, since the infectious disease burden in the Global South is high, banning or restricting the use of AGP without effective alternatives would impair bird health, bird welfare, food and nutrition security, and human health (Thema et al., 2019). Antibiotic-free broiler production has been linked to negative impacts on bird health, welfare, production efficiency, product quality, and costs (Cervantes, 2015). To maintain consumer acceptance, researchers and producers must identify and assess AGP alternatives that promote gut eubiosis for optimal growth and product quality. These alternatives

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should also support economically, environmentally, and socially sustainable poultry production. In this regard, nucleotides and probiotics have gained attention as potential AGP replacements, including in layer chickens. For many years, probiotics and nucleotides have been separately touted as potential AGP alternatives that can support eubiosis in the gut of intensively reared chickens. However, there are several parameters that need to be optimized and challenges that need to be addressed for them to effectively replace AGPs (Popova, 2017) and generate consistent results. The popularity of nucleotides and probiotics as alternatives to AGPs is based on evidence of their individual effectiveness in a range of animal models. Probiotics can reduce the abundance of harmful bacteria while increasing beneficial bacteria such as *Lactobacilli* and *Bifidobacteria*, leading to improved digestion and nutrient absorption (Patterson and Burkholder, 2003). Probiotics can also directly interact with the host immune system, stimulating the production of immunoglobulins and enhancing the activity of immune cells (Yazhini et al., 2018). By comparison, nucleotides play a critical role in cell growth, development, and repair, including gut and microbial cells (Sauer et al., 2011). During periods of rapid growth, stress, or immune system challenges, animals may require higher levels of nucleotides, as endogenous synthesis may be insufficient to meet their metabolic demands (Maldonado et al., 2001). Under such conditions, nucleotides can become conditionally essential nutrients (Stein and Kil, 2006). Supplementing such animals with nucleotides can improve growth, immune function, and disease resistance. This approach could be important in intensively reared poultry because maize-soybean diets are typically nucleotide deficient (Mateo and Stein, 2004).

Given that the mechanisms of action for probiotics and nucleotides in chickens appear to complement each other, it is logical to anticipate a synergistic effect when the two additives are used simultaneously. However, this supplementation strategy has not been investigated in poultry including layer chickens, possibly because the synergistic mechanisms have neither been proposed nor validated. Accordingly, this review proposes hypothetical synergistic mechanisms of nucleotide-probiotic blends, explaining how these could lead to consistent outcomes and enhance the value of these additives as AGP replacements.

Layer chicken production

Globally, layer chicken production is one of the most important agricultural sectors due to its role in human nutrition, economics, and livelihoods. The industry contributes significantly towards the economic growth and food and nutrition security of many countries (Borda-Molina et al., 2021). Despite, the positive contributions to human livelihoods, health, and nutrition, layer chicken production still faces diverse production, management, economic, and health challenges. Gautron et al. (2021) identified welfare and sustainability issues; the fate of culled male day-old chicks; increased financial cost and logistics due to *in ovo* sexing; and increased cost of eggs due to adoption of cage-free production systems, as the major issues facing the table egg industry. A study by Castellini et al. (2012) classified these challenges into environmental (e.g., increased recourse to natural resources, pollution, etc); economic (e.g., higher costs of conventional inputs); and social sustainability (poor product quality and safety, suboptimal bird welfare, etc) problems. Most of the identified challenges arise due to the pressure that producers face to meet the rapidly rising demand for eggs (Tarawali et al., 2011; Ravindran, 2013; Yitbarek and Berhane, 2014).

Intensive production systems have become the main source of eggs despite the attendant disease risks (Zaheer, 2015; Andam et al., 2017). Intensification of layer chicken production systems has reduced the acceptability of eggs by consumers concerned about suboptimal bird welfare and compromised product quality. In these egg production systems, layers are kept in cages that limit their freedom of movement and ability to express natural behaviours (Hartcher et al., 2017). Such highly stressful production systems result in feather pecking, keel bone damage, foot pad lesions, high mortality, and compromised hen health (Meseret et al., 2016; Singh and Grooves, 2020). Traditionally, the effects of stress in these systems have been managed through the use of AGPs (Zaheer, 2015). However, the use of AGPs in poultry production systems has been identified as one of the drivers of antimicrobial resistance in human and animal pathogens (Harper and Makatouni, 2002; Zaheer, 2015; Tenrisanna and Rahman, 2023). Cage-free production systems have also been used to reduce housing-induced stress in layer chickens. However, production outputs have been reported to be poor (Harper and Makatouni, 2002) while cage-free eggs command a premium price in the market, with some studies suggesting that they cost twice the price of conventional eggs (Pelletier et al., 2018). The high cost of cage-free eggs may negatively affect the viability and profitability of this production system.

Stress in intensive layer chicken production systems

Stress negatively influences the performance, health, welfare and product quality in layer chickens. Stress caused by high stocking density has been shown to compromise feed intake, productivity, welfare, and product quality in poultry birds. Hedlund and Jensen (2022) found that overly stocked White Leghorn layer chickens experienced more feather pecking damage, lower hatch weight, and produced smaller eggs compared to birds raised under optimal conditions. The lower hatch weight could be triggered by increased competition for nutritional resources. Feather pecking occurs when hens redirect feeding behaviour to removing the feathers of other hens (Newberry et al., 2007). Extensive and persistent feather pecking can cause loss of animal stock through cannibalism and death, which results in significant economic losses (Cronin and Glatz, 2020). Some studies have suggested that high stocking densities reduce eggshell thickness and strength in laying ducks (Xiong et al., 2020) and quail (Aro et al., 2021). The reduction in eggshell thickness and strength may be due to reduced absorption and utilization of calcium in the gut. Furthermore, eggs in birds reared under high stocking density tend to be smaller in size and weight compared to eggs from birds reared under optimal conditions. High stocking densities also cause radiant heat to increase between birds and causes the temperature to increase (Wasti et al., 2020). As a result, birds may fail to efficiently dissipate their body heat due to high metabolic rates and reduced airflow (Wasti et al., 2020).

In hot environments, densely stocked layer chickens tend to consume less feed because their priority is to dissipate as much heat as possible from their body (Oluwagbenga et al., 2023). Feed avoidance is also designed to prevent overheating due to heat increment of nutrient digestion and metabolism (Oluwagbenga et al., 2023). The reduction in production performance-related parameters could, therefore, be attributed to reduced feed consumption triggered by these thermoregulatory mechanisms. He et al. (2018) proposed that the effects of heat stress in poultry birds are mediated through the activation of the hypothalamic-pituitary-adrenal axis, elevated corticosterone concentrations and mitochondrial activity, and stimulation of central nervous system, which reduces daily gain, feed intake, and feed conversion ratio (FCR) in poultry. Indeed, Deng et al. (2012) demonstrated that layer chickens reared at 34 °C had lower feed intake, depressed egg production, elevated corticosterone levels, and compromised immune function. Furthermore, heat stress has been reported to compromise the number and size of follicles, particularly the yellow and hierarchal follicles (Li et al., 2020; Ghoname et al., 2022) resulting in compromised egg quality (internal, external and reproductive capacity). The size and number of follicles serve as an indication of the reproductive/fertilization capacity of an egg. In addition, the decrease in egg quality is also caused by changes in the blood chemistry of the layer chicken and low calcium absorption rates in the small intestines (Barrett et al., 2019).

Antibiotics as mitigators of stress in layer chickens

Applications

To meet the rising demand for eggs from a growing human population, the layer chicken industry employs large-scale, industrialized chicken farms. These production systems impose a wide range of stressors on chickens resulting in gut dysbiosis, poor immune function, and proliferation of pathogenic microorganisms. To mitigate against the negative impact of stress in intensive layer chicken farms and avert production losses, subtherapeutic in-feed antibiotics have been traditionally used (Lima et al., 2023). Indeed, Engberg et al. (2000) confirmed the efficacy of in-feed prophylactic antibiotics in reducing stress-induced disease outbreaks and improving feed utilization efficiency in intensive poultry production systems.

Bird performance, health, welfare, and egg quality outcomes

Although several studies have reported positive performance outcomes of broilers when antibiotic growth promoters have been used (Engberg et al., 2000; Dumonceaux et al., 2006; Miles et al., 2006), there are very few such reports on layer chickens. To complicate matters, studies on layers have yielded inconsistent results. For instance, Dipeolu et al. (2005) found that adding tetracycline and the enzyme Avizyme 1500® at 200 mg/kg to the basic diet improved FCR, final body weight, and weight gain in Nera pullets. In contrast, Wu et al. (2008) reported that supplementing the diets of Bovans White and Dekalb White layer hens with 33.7 g/ton of the antibiotic tylosin had no effect on feed intake, feed conversion efficiency, or body weight. These discordances can be attributed to factors such as the specific antibiotics used, diet composition, bird species, and environmental conditions.

Antibiotics possess antimicrobial activity that prevent infectious bacterial diseases, resulting in better bird health (Khalifeh et al., 2009). Jankowski et al. (2022) reported that adding 10 % enrofloxacin at a dose of 0.5 mL/L of drinking water enhanced the bursa of Fabricius and bursa of Fabricius index of the broilers compared to the control. In birds, the spleen, thymus, and bursa of Fabricius are vital organs for humoral and cellular immunity. The bursa of Fabricius is the primary lymphoid organ where B cell proliferation and differentiation occur (Glick et al., 1994; Jankowski et al., 2022). Bursa of Fabricius index, on the other hand, measures the effect of various infectious and non-infectious factors on the function of the bird's immune system (Ellakany et al., 2008). So, in the presence of immunosuppressive factors, the growth bursa of Fabricius will be inhibited (Yin et al., 2015), and bird health might suffer. Dumonceaux et al. (2006) noted that the addition of virginiamycin (20 g/ton) as a growth promoter in broiler's diets enhanced the abundance of *Lactobacillus* species in the duodenal loop at proximal ileum compared to control-fed birds. *Lactobacillus* species produce bacteriostatic bacteriocin-like compounds and lactic acid, which play a major role in lowering the gut pH. Additionally, antagonism and competitive exclusion have been proposed as processes by which lactobacilli species inhibit the multiplication of pathogenic bacteria and regulate the gut flora (Servin, 2004). Accordingly, an increase in *Lactobacillus* species in the gut is associated with improved gut health of the birds. However, Engberg et al. (2000) reported that when salinomycin (0.06 g/kg feed), zinc bacitracin (0.02 g/kg feed) or their combination were added in broiler diets, the population of *Clostridium perfringens* and *Lactobacillus* species in the intestinal contents of broilers were lowered compared to antibiotic-free feed. It is worth noting that there is no reported evidence on the impact of antibiotics on the immunity of layer chickens, hence inferences in this review have been drawn from broiler chickens.

Currently, the poultry sector needs to transition towards antibiotic-free production, both to combat the development of antibiotic resistance and to meet market demands for antibiotic-free poultry products. However, without suitable alternatives, this strategy could be detrimental to the health and welfare of birds (Iannetti et al., 2021) as well as product quality and safety. Indeed, Karavolias et al. (2018) noted that eliminating antibiotics can lead to a higher risk of specific diseases, contradicting widely held beliefs that the elimination of antibiotics supports good animal welfare. Similarly, a study by Li et al. (2020) observed a growing trend of rearing chickens without antibiotics might severely impact the health and welfare of the animals, especially increasing disease incidence and mortality. Further research is needed to identify and standardize a set of managerial approaches to improve the health of poultry birds reared without antibiotics. This review proposes the use of probiotics and nucleotides to aid the transition to AGP-free layer chicken production.

Over the past few decades, the use of antibiotics as growth promoters in layer diets has been discouraged in several countries, with the European Union (EU) banning their inclusion in animal feed (Dipeolu et al., 2005). This prohibition stems from concerns about antibiotic residues in poultry products, such as eggs, which pose potential health risks to consumers (Kümmerer, 2009; Ronquillo and Hernandez, 2017). Indeed, Dipeolu et al. (2005) reported that supplementing a diet with tetracycline resulted in residues in the eggs of laying birds. Several scholars have also reported the presence of trace antibiotics in commercial eggs (Ben et al., 2022; Ma et al., 2022). However, no effects of antibiotics were recorded on egg quality parameters such as egg shape index, yolk colour, egg composition values and blood spots. Similarly, Wu et al. (2008) discovered that tylosin supplementation had no effects on egg solids but reduced the proportion of dirty eggs, resulting in improved egg quality of Bovan White and Dekalb White hens.

Contemporary challenges of antibiotic use

Antibiotic use in layer chickens threatens the safety of eggs (via antimicrobial residues) while driving the development and spread of microbial resistance (Agyare et al., 2018). Owusu-Doubreh et al. (2023) reported that in-feed antibiotics that are used mitigate stress effects can remain in poultry products including eggs, compromising food safety and posing a risk to human health. Indeed, Velazquez-Meza et al. (2022) reported that antibiotic residues in food can result in allergic reactions and even contribute to the development and spread of antimicrobial resistance. In chickens, antibiotics can be transferred from parents to chicks by vertical transmission (Jansen et al., 2020). As the chick grows, the concentration of antibiotics in the body progressively declines as they are eliminated through faeces, a common source of trace antibiotic contamination on feeders, drinkers and walls (Lima et al., 2023). To prevent product contamination and the spread of antimicrobial resistance, researchers have been searching for functional alternatives to antibiotics, such as nucleotides and probiotics.

Alternative mitigators of stress

Consumer pressure and concerns about the harmful effects of antibiotic use, as well as the antibiotic ban by some countries, have prompted researchers to consider alternatives to antibiotics (Diarra and Malouin, 2014). These alternatives should be able to enhance growth metrices, health and egg output, as well as reduce the incidence of stress-induced diseases in layer chickens while preserving environmental and consumer health. Current research focuses on natural alternatives with benefits comparable to those of antibiotics (Mehdi et al., 2018). Nucleotides and probiotics are some of the candidates that have garnered interest as antibiotic alternatives due to their potential to optimize the gut environment, reduce oxidative stress, and boost immunity and growth performance in chickens.

Nucleotides as feed additives in layer chicken production

Definition and applications

Nucleotides are low molecular weight compounds that are derived from nucleic acid hydrolysis and comprise of a nitrogenous base (pyrimidine or purine), which is linked by a pentose sugar (ribose and deoxyribose) and a two or three phosphate group (Sauer et al., 2011; Preller and Manstein, 2017; Lawrence, 2022). Nucleotides are found in many food sources in the form of nucleoproteins and nucleic acids (Dancey et al., 2006). However, soy, vegetables, and dairy products have a lower concentration of nucleotides compared to mushroom, fishmeal, legumes, animal protein soluble and yeast extracts (Ding et al., 2021). In theory, animals may require higher nucleotide levels during periods of rapid growth, stress, or immune system compromise (Stein and Kil, 2006), when endogenous synthesis is insufficient (Maldonado et al., 2001). Accordingly, it may be cost-effective to supplement nucleotides only during the periods when the bird has a higher requirement such as during the transition between growing/production stages when diets are changed. Studies suggest that nucleotide supplementation can improve animal performance under stressful conditions by modulating local immune response and reducing inflammation (Onrust et al., 2015; Valini et al., 2021). Reduction of disease incidences in nucleotide supplemented chickens is linked to the predominance of *Lactobacilli* in the gut microflora (Carrey et al., 2013) and maintenance of the T receptor cells in the gut (Ding et al., 2021). Furthermore, nucleotides also improve immunity of animals by altering intestinal structure and innate and adaptive immune responses, thus increasing stress tolerance (Baines and Brown, 2016). There is evidence that nucleotides result in the transfer of desirable gut microbiota to products, thus could improve the quality and safety of foods for consumers in layer chickens (Tapingkae et al., 2018; Liu et al., 2021). The influence of dietary nucleotides on bird health, welfare, gut health, and product quality as well as the associated mechanisms are summarized in Table 1.

Mechanisms of action

Modification of gut microbiota and local environment

Nucleotide supplementation has been shown to improve bird productivity and welfare and product quality by modifying the gut microbiota, suppressing inflammation, and improving immune responses (Wu et al., 2018; Mohamed et al., 2020; Liu et al., 2021). It has been reported that poultry birds offered diets fortified with nucleotides have a more diverse gut microbiota compared to those on conventional diets (Kruger and Werf, 2018). A diverse and rich gut microflora is desirable because it plays a critical role in enhancing intestinal barrier function (Mindus et al., 2021). Several studies have demonstrated that chickens supplemented with nucleotides have a higher concentration of gram-positive bacteria (*Bacilli, Lactobacillales*, and *Enterobacteriales*) compared to gram-negative bacteria (*Blautia, Ruminoclostriduium, Sellimonas, Eisenbergiella, Escherichia-Shigella*) (Wu et al., 2018; Liu et al., 2021; Zhen et al., 2021). Gram-positive bacteria such as *Lactobacillus* lower the intestinal pH by producing organic acids such as lactic and propionic acids, creating a favourable environment for the proliferation of beneficial microbiota and reducing harmful microbiota (Wang et al., 2023). Therefore, nucleotides indirectly modify the host's local environment by reducing the intestinal pH (Kreuz et al., 2020).

Intestinal barrier function

Nucleotides have been shown to enhance the epithelial barrier function by proliferating enterocytes, mucosal protein, DNA, and increasing villi height (Kreuz et al., 2020; Mohamed et al., 2020; Aziz et al., 2024). Furthermore, nucleotides also code for genes that are used for synthesis of protein and protection of cell structures (Mohamed et al., 2020). They also upregulate the production of immunoglobins, which bind antigens and hinder them from passing through the intestinal mucosal membrane (Aziz et al., 2024). Components of nucleotides, pyrimidines, adenosine, uridine, and cystine improve the digestive capacity of the proliferating enterocytes and lymphocytes as well as the synthesis of RNA in the jejunum crypts (Aziz et al., 2024). This is crucial for enhancing immune response, glutathione metabolism, vitamin transport and metabolism, lipid metabolism, as well as neuronal and cardiac development, maturation, and growth (Sinpru et al., 2021).

Larger villi increase the amount of nutrients that an animal can digest and absorb within a given timeframe (Kamel et al., 2021). Hence, some studies have reported that chickens supplemented with nucleotides had a higher feed intake and digestibility compared to control birds (Kamel et al., 2021). In turn, higher feed intake translates into improved egg production (Qiu et al., 2024). The increased feed intake could also be attributed to enhanced microbial function, which promotes the production of SCFAs, upregulates digestive enzymes, increases mucosal development, enlarges villi size and crypt depth, and boosts goblet cell production (Wu et al., 2018; Mohamed et al., 2020; Kamel et al., 2021,). Increased feed intake and bird productivity could also be attributed to inherent components of the nucleotide mix such as oligosaccharides, peptides, and amino acids, which can increase appetite and stimulate GIT development.

Table 1
The effects of dietary nucleotides on health, welfare, productivity, and product quality in layer chickens and their associated mechanisms of action.

Nucleotide Type	Strain	Outcomes	Possible mechanisms of Action	Refs.
Yeast culture	Hy-line brown Layers	• Improved egg quality, hatching rate, ileum fat digestibility, antioxidant capacity, serum lysozyme • Reduced aspartate aminotransferase activity	• Increase in relative proportions of Lactobacillus and Bacilli. • Enhanced lipopolysaccharide biosynthesis, glutathione metabolism, and ubiquinone metabolism	Liu et al. (2021)
Yeast culture	Hy-line brown Layers	• Improved villus height, egg production and egg quality. • Enhanced serum antioxidant capacity and glutathione peroxidase activity	• Higher population of Bacteroidetes • Lower population of Proteobacteria	Qiu et al. (2024)
Brewer's yeast hydrolysate	Hy-line brown Layers	• Improved egg production, internal egg quality, and eggshell thickness • Decreased *Lactobacillus* and *E. coli* strains	• Enhanced digestibility of fibrous feeds by breaking them down into their smaller constituents. • Hydrolysate contains proteins, nucleotides, mannan-oligosaccharide and B-glucan. Prevented pathogens from attaching and binding to the villi	Park et al. (2020)
Dietary red yeast	Esa brown Layers	• Increased feed efficiency, egg yolk colour score and caecal short chain fatty acids • Reduced yolk cholesterol and triglyceride • Reduced hepatic hydroxymethylglutaryl-coenzyme A reductase activity	• Bright egg yolk colour due to pigments in red yeast • Low cholesterol and triglycerides due to elevated levels of Saccharomyces, which remove cholesterol from the gut • Increase in caecal short chain fatty acids due to mannan-oligosaccharides mechanism and elevated nutrient utilization	Tapingkae et al. (2018)
Yeast β-glucan	Hy-line brown layers	• Increased the relative abundance of *Bacilli, Lactobacillales*, and *Enterobacteriales* and metabolic profiles of the laying birds.	• Mechanism unknown	Zhen et al. (2021)
Enzyme-treated yeast	Lohmann LSL Lite layers	• Reduced number of damaged and cracked eggs • Increased eggshell quality, egg weight and plasma protein and albumin	• Modified amino acids metabolism • Increased dietary calcium absorption, hence improved eggshell quality.	De Cloet et al. (2023)

Inflammation and immune responses

Nucleotides also modulate bird health and welfare through cell-mediated immunity (Mohamed et al., 2020). Nucleotides increase the relative proportion of immunoglobins to protect the host against pathogens (Kreuz et al., 2020; Mohamed et al., 2020). Furthermore, nucleotides modulate bird welfare by regulating the expression of anti-inflammatory and pro-inflammatory biomarkers (Kreuz et al., 2020; Aziz et al., 2024). Aziz et al. (2024) found that nucleotides upregulated antioxidant biomarkers by promoting synthesis of mRNA, resulting in the production of enzymes that combat oxidative stress. In addition, nucleotides conserve glutamine, aspartate, and glycine for *de novo* synthesis, resulting in the use of these amino acids for growth (Kreuz et al., 2020). Increased glutamine availability is especially crucial for high-glutamine-demand processes and organs, such as the pectoralis major muscle, which contains 0.06 g of glutamine per gram (Hu et al., 2023).

The enhanced immunity and intestinal mucosal functions of layer chickens fed nucleotides is also associated with regulation of inflammatory biomarkers and genes (Wu et al., 2018). Nucleotides have been demonstrated to increase the expression of anti-inflammatory genes (INF-γ, IGF Insulin growth factors, IL-1β, IL-2, IL-6, IL-10, mucin (MUC2), and TTF2) and reduce the expression of pro-inflammatory genes (IL-17A, IL-22 and IFN-a genes (Wu et al., 2018; Mohamed et al., 2020; Kamel et al., 2021; Rady et al., 2023; Ismail et al., 2024). Mucin (MUC2) can reduce the expression of pro-inflammatory genes or incidences of inflammation (Mohamed et al., 2020). Interleukin-6 provides short-term protection against infection or damage by alerting the immune system to the source of inflammation, whilst IL-1β plays a critical role in regulating expression of inflammatory responses (Ismail et al., 2024). Interleukin-10 and IGF prevent tissue damages by reducing inflammatory and autoimmune pathologies (Rady et al., 2023). However, some studies report that dietary nucleotides could not modulate the expression of IL-1β, a pro-inflammatory cytokine, and IL-10, an anti-inflammatory cytokine in broiler chickens (Leung et al., 2019), suggesting that influence of nucleotides on inflammation may be gene-dependent or could be modulated by experimental conditions. Mucin is a key component of the protective mucus layer, which safeguards the intestines from pathogen invasion, thereby preserving intestinal integrity (Liévin-Le Moal and Servin, 2006; Cornick et al., 2015).

Given the positive effects of nucleotides on the bird's immune system, it is not surprising that their dietary supplementation would result in lower mortality rates. Indeed, Hassanein et al. (2023) found that incorporation of nucleotides into the soybean meal diet reduced the mortality of broiler chickens. Similarly, Rutz et al. (2007) found that nucleotides reduced the mortality rate of stressed chickens by 30 %. It has been suggested that nucleotides boost the survival rates of chickens by enhancing energy storage and immune responses during growth and development (Rutz et al., 2007; Kamel et al., 2021; Hassanein et al., 2023). The improved survival rates of the poultry birds has also been attributed to mechanisms such as cell-mediated immunity and resistance to bacterial infections (Mohamed et al., 2020). The reduction in mortality in birds supplemented with nucleotides is also thought to be mediated through reduction in stress hormones (Kamel et al., 2021; Hassanein et al., 2023).

Probiotics as additives in layer chicken production

Definition and applications

Gadde et al. (2017) reported that probiotics include a diverse range of species categorized as *Lactobacillus, Bacillus, Bifidobacterium, Lactococcus* species, Gram-positive cocci, *Streptococcus*, and yeast (*Saccharomyces*) genera, with growth-promoting potential, non-toxic side effects, health benefits, and other characteristics. Indeed, several authors have reported improved performance parameters, including feed conversion ratio, egg quality, egg production, disease resistance, and overall animal welfare when layer chicken were supplemented with probiotics (Sjofjan et al., 2021; Bindari and Gerber, 2022; Jiang et al., 2022). This suggests that probiotics can be a viable alternative to antibiotics because they do not contribute to AMR or unsafe poultry products. Their diverse functions in enhancing animal, human and environmental health stem from their ability to control and suppress pathogens, reduce toxins and pollutants, and increase nutrient bioavailability (Oelschlaeger, 2010) through three primary mechanisms of action. According to Kouhounde et al. (2022), these mechanisms of action include competition with pathogens for surface attachment and nutrients, facilitated by macromolecular structures in the cell wall (e.g., exopolysaccharides and S-proteins) and secreted amphiphilic compounds (e.g., biosurfactants); antimicrobial production (e.g., antioxidant compounds bacteriocins, enzymes and antiviral agents); and immunomodulation activity on immune cells. These mechanisms of action are discussed next.

Mechanisms of action

Whereas several milestones have been achieved in the study of probiotics, their modes of action still require further elucidation (Latif et al., 2023). Their numerous beneficial effects on poultry production and health have been attributed to several underlying interconnected mechanisms of action that are summarized in Table 2. Probiotics produce antimicrobial substances such as acidophillin, organic acids, lactocidin, bacteriocins, and hydrogen peroxide resulting in the reduction of viable counts of pathogenic bacteria in the gut (Fantinato et al., 2019; Ahire et al., 2021). Volatile fatty acids and organic acids are produced from the natural breakdown and metabolism of feed components by probiotics in the gastrointestinal tract. This results in a reduction in gut pH to a level that is unsuitable for the survival of pathogenic bacteria such as *Escherichia coli* and *Salmonella* species (Khan and Naz, 2013). Furthermore, probiotics enhance the intestinal barrier function by stimulating the synthesis of mucin proteins (Zhao et al., 2021), influencing the expression of tight junction proteins such as claudin-1 and occludin, and modulating the gut immune response. Gou et al. (2022) stated that the intestinal barrier function consists of the physical, chemical, immune, and microbial barriers, which play a significant role in the defense against infections, toxins, antigens, and other harmful substances. Physical and chemical barriers are important in regulating intestinal permeability and preventing intestinal epithelial cell damage as well as bacterial translocation. The intestinal microbial barrier comprises of symbiotic microorganisms in the gut that help inhibit pathogens proliferation by competing for limited nutrients and releasing antimicrobial substances. The immune barrier plays a crucial role in preventing pathogen invasion. Additionally, these microorganisms support the host with various physiological processes (Takiishi et al., 2017).

Another mechanism of action is through competitive exclusion Chichlowski et al. (2007), where probiotics colonize adhesion sites on the intestinal epithelium, preventing pathogenic bacteria from forming colonies. Probiotics also enhance digestion by increasing the activity of digestive enzymes as well as nutrient absorption while decreasing the activity of pathogenic bacterial enzymes (glucuronidase, azoreductase, and nitroreductase) (Chowdhury et al., 2020). Probiotics can also regulate both the innate and adaptive immune systems by modulating dendritic cells, T lymphocytes, and macrophage B, as well as enhancing the production of γ-interferon (Latif et al., 2023). This probiotic action enhances the host immune system's ability to respond effectively, thus preventing the colonization of gut by harmful microbes (Ayana and Kamutambuko, 2024). Probiotics also stimulate the production of anti-inflammatory cytokines, interact with intestinal epithelial cells, and attract mononuclear cells and macrophages (Yang et al., 2009; Petruzziello et al., 2023). These actions maintain a balanced immune response by preventing excessive inflammation, which would otherwise compromise gut health. Furthermore, probiotics positively modify intestinal morphology by increasing villus height, goblet cell population,

Table 2
Effects of probiotics on health, welfare, productivity and product quality in chickens and their associated mechanisms of action.

Probiotic type	Outcomes	Mechanisms of action	Refs.
Commercial probiotic (Protexin™) containing Lactobacillus *plantarum, Lactobacillus delbruecki subsp. bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus salivarus subsp. thermophiles, Enterococcus faecium, Aspergillus oryza* and *Candida pintolopesii*	Enhanced both egg weight and egg mass of Hisex Brown layers.	• Increased retention of nutrients, including calcium, nitrogen, fat, and phosphorus.	Balevi et al. (2001)
Metabolites from *Lactobacillus plantarum*	Enhanced feed utilization efficiency, body weight and weight gain of broilers.	• The metabolites have bacteriostatic and bactericidal effects, which reduced pathogenic bacterial load in the gut microflora.	Thanh et al. (2009)
Clostridium butyricum	Quadratic increase in *Bifidobacterium* and reduction in *Escherichia coli* counts in Jinghong-1 strain laying hens.	• Production of large amount of SCFA by *Clostridium butyricum*. • Probiotic also competed with pathogens for attachment sites on the gut surface.	Zhan et al. (2019)
Bacillus licheniformis DSM5749	Enhanced egg production rate and average daily egg yield of Hy-Line Brown laying hens.	• Beneficial metabolites by *B. licheniformis*, such as extracellular digestive enzymes, lysozyme, various antibiotics, and antifungal proteins. • Increased lipase activity resulted in improved laying performance observed.	Pan et al. (2022)
Multi-strain probiotic	Improved albumen weight, yolk height, yolk length, and yolk index during storage of eggs from Hyline W36 lineage hens.	• Higher protein deposition in the eggs. • Beneficial modulation of the intestinal microbiota, which offered better health and, as a result, improved digestion and absorption of nutrients. • Higher yolk index linked to hepatocytes synthesizing vitellogenin, a protein that transports lipids from the liver to the developing oocytes that produce the yolk.	Carvalho et al. (2022)

and decreasing crypt depth, resulting in a favorable environment for the gut microbiota and overall host health. Indeed, Ding et al. (2021) reported that probiotics improve digestion, nutrient absorption, gut health, and immune response and inhibit pathogenic intestinal bacteria in chickens.

Potential nucleotide–probiotic synergism

Given that the discussed mechanisms of action for probiotics and nucleotides in chickens are related and appear to complement each other (Fig. 1), it is logical to anticipate synergistic effects (Table 3) should the two additives be fed together. Both additives have been shown to support gut health and boost immune function in chickens when used individually (Fig. 1). In combination, probiotics and nucleotides could better modulate gut microbiota, as nucleotides have been shown to promote the growth of beneficial bacteria such as *Lactobacilli* (Sauer et al., 2011), while probiotics have been reported to reduce the abundance of potentially harmful bacteria (Yazhini et al., 2018). The nucleotide-probiotic blend is likely to promote a healthy gut microbiota, resulting in improved digestion, nutrient absorption, and immune function (Fig. 2). In addition, both additives demonstrate complementary anti-inflammatory properties, which could better reduce the negative effects of stress on the gut and immune system. By reducing inflammation, these additives can help to improve gut health and immune function, leading to better performance in chickens. Furthermore, nucleotides are important building blocks for DNA and RNA (Sauer et al., 2011), while probiotics have been shown to promote balanced gut microbiota and enhance the activity of immune cells, specifically T cells (Popova, 2017). This combined effect can help to support cell growth, development, and repair, as well as improve the bird's ability to fight off infections.

Overall, the potential for synergistic outcomes when feeding probiotics and nucleotides to chickens is high, as both additives have complementary effects on gut health, immune function, and performance (Figs. 1 and 2). In combination, these additives can provide a more comprehensive and effective approach to improve the health and productivity of chickens reared under highly stressful intensive production systems. By understanding the interactions between dietary nucleotides, probiotics, and the gut microbiome, it may be possible to develop more effective supplementation strategies for improved gut health, growth performance, and egg quality in chickens. The mechanisms that could be involved in these potential synergistic effects on gut function, bird health, and bird productivity are summarized in Figs. 1 and 2 and discussed below.

Gut function

Modification of gut microbiota

The mechanisms of action of both dietary nucleotides and probiotics in poultry are closely linked to their effects on composition of gut microbiota (Wu et al., 2018; Kamel et al., 2021). Studies have shown that layers exhibiting favorable production and egg quality traits possess a diverse microbiota, rich in gram-positive bacteria (Rutz et al., 2007; Kamel et al., 2021; Obianwuna et al., 2022; Hassanein et al., 2023). Beneficial gut microbiota triggers secondary metabolic and physiological processes essential for the bird's well-being and contributes to the production of high-quality eggs (Kamel et al., 2021; Liu et al., 2021;

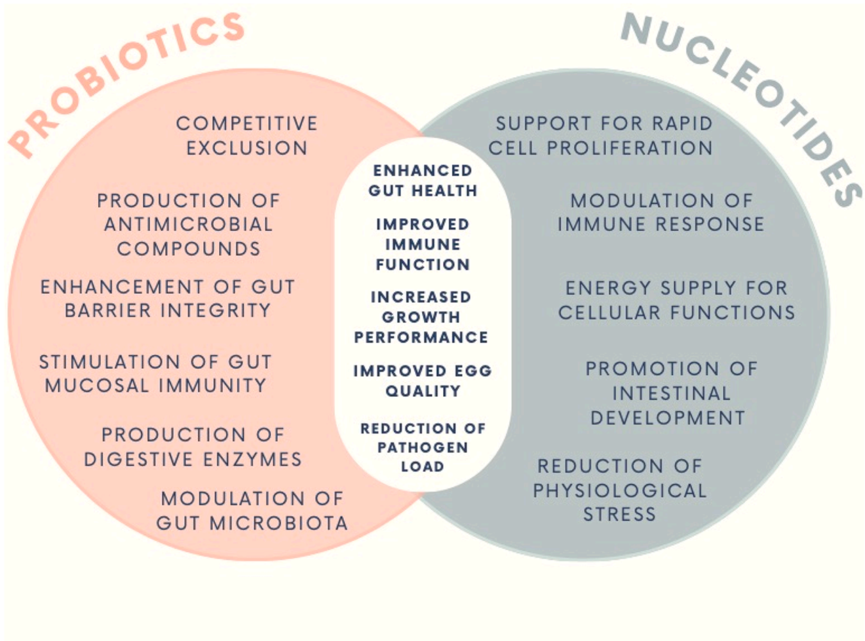

Fig. 1. Distinct and shared mechanisms through which nucleotides and probiotics enhance bird performance and product quality.

Niyishama et al., 2021; Xu et al., 2023; Qui et al., 2024). Nucleotides could modulate gut microbiota by promoting growth of gram-positive bacteria (Wu et al., 2018; Ismail et al., 2024), whilst probiotics reduce the relative abundance of gram-negative bacteria (Khan et al., 2020; Xu et al., 2023). Since both nucleotides and probiotics can modulate gut microbiota, it can be suggested that their combined use may have a synergistic effect, significantly enhancing hen productivity and product quality. The augmentation of gut microbiota will result in improved egg quality, bird welfare, gut health and production performance.

Structure and epithelial barrier function

A well-developed and functional intestinal environment is essential for the optimal growth and performance of birds, given its critical role in metabolic and physiological processes (Tang et al., 2020). High stocking densities can lead to underdeveloped intestines due to reduced feed intake, chemical imbalances, and decreased mucosal production (Mashaly et al., 2004; Deng et al., 2012; Li et al., 2020). Nucleotides and probiotics have both been shown to enhance the structure and functionality of the gastrointestinal tract in poultry by modulating the gut microbiota and increasing villus height (Qui et al., 2024). Nucleotides improve the GIT barrier function by promoting the proliferation of enterocytes, mucosal proteins, and DNA (Kamel et al., 2021; Mohammed et al., 2020), while probiotics modulate and protect the gut microbiome (Odenwald and Turner et al., 2017). The production of a thicker mucosal layer and the proliferation of enterocytes help prevent pathogen infiltration into the intestines (Li et al., 2020). Additionally, nucleotides and probiotics improve epithelial function by increasing the population of *Lactobacillus*, which helps to scavenge pathogens attached to the epithelial surface (Mohammed et al., 2020; Thanh et al., 2009). Therefore, a nucleotide-probiotic composite in poultry could enhance epithelial barrier function through these complementary actions.

Bird health

The modulation of the gut microbiota significantly impacts immune health and the expression of specific cells and genes. Incorporating nucleotides and probiotics into poultry diets has been shown to reduce the H:L ratio and the expression of pro-inflammatory and stress-related biomarkers in poultry birds. Nucleotides exert their effects by modulating the expression of these genes and biomarkers through cell-mediated immunity (acting as immunomodulators) and increasing synthesis of mRNA (Mohammed et al., 2020; Aziz et al., 2024), while probiotics could complement nucleotides by restoring the equilibrium of the gut microbiota, mainly *Lactobacillus*, and enhance intestinal antioxidant activities (Thanh et al., 2009). Indeed, probiotics enhance the activities of T-SOD, GSH-Px, CAT, and glutathione transferase enzymes, thereby reducing malondialdehyde levels in poultry birds (Wang et al., 2022). These enzymes play a pivotal role in the gut as antioxidants, safeguarding the intestines from reactive oxygen species (ROS) (Bhattacharyya et al., 2014). Malondialdehyde, on the other hand, diminishes the activities of digestive, absorption, and antioxidant enzymes, impairing gastric and intestinal mucosa, which are essential for optimal nutrient absorption and utilization in the gastrointestinal tract (GIT) (Fan et al., 2023). This reduces the quantities of nutrients that the birds can access to meet their biological, metabolic, and physiological processes (Fan et al., 2023). Furthermore, the types of anti-inflammatory biomarkers/genes expressed by the additives have been shown to vary and could complement each other. Nucleotides enhance the expression of INF-y, insulin growth factors, IL-1β, IL-2, IL-6, IL-10, mucin (MUC2) and TTF2 whilst probiotic upregulates the expression of ovarian tissue (toll-like receptor-4) (Wu et al., 2018; Mohammed et al., 2020; Kamel et al., 2021; Rady et al., 2023). In terms of animal welfare, nucleotides reduce incidents of feather pecking by increasing the population of *Lactobacillus* in the gut, increasing expression of T-cells, and reducing caecal microbiota whilst probiotics regulates/modulates the expression of regulatory T cells (Mindus et al., 2021).

Bird productivity

Both probiotics and nucleotides enhance poultry productivity by upregulating digestive enzymes resulting in increased feed utilization and intake as well as bird survival rates. Probiotics improve nutrient digestion (Xu et al., 2003) while nucleotides play a critical role in tissue (including intestinal) growth and repair (Aziz et al., 2024; Kreuz et al., 2020). Therefore, it is conceivable that combining probiotics and nucleotides in poultry diets can significantly boost chicken productivity by enhancing digestive enzyme activity, nutrient absorption and utilization

Table 3
Potential synergy between nucleotides and probiotics as derived from their reported individual effects and mechanisms of action.

Parameter	Outcomes and mechanisms		Potential synergistic action
	Nucleotides	Probiotics	
Growth performance	• Reduction of mortality of chickens and increasing survival rates. • Improved feed intake by increasing size of the villi, augmenting gut microbiota, increased SCFA production and upregulating digestive systems. • Increased the strength of the eggshell by increasing the utilization and absorption of calcium in the GIT	• Reduced mortality rates in chickens, thus improving their survival rates. • Improved weight gain by secreting digestive enzymes that might improve the digestion rate of nutrients.	Probiotics and nucleotides increase feed intake, survival rates, feed utilization efficiency, and growth of birds. Probiotics increase the digestion of nutrients whilst nucleotides play a critical role in the growth and repair of gut and general body cells. These actions can complement each other to produce better responses in layer chickens.
Bird health	• Reduced H:L ratio and corticosterone. • Upregulate anti-inflammatory biomarkers (INF-y, IGF Insulin growth factors, IL-1β, IL-2, IL-6, IL-10, mucin (MUC2) and TTF2) and reduce the expression of pro-inflammatory biomarkers (IL-17A, IL-22 and IFN-a genes). • Enhanced bird health by increasing cell-mediated immunity and resistance to bacterial infections. • Upregulate antioxidant biomarkers by increasing the synthesis of mRNA. • Increase the relative proportion of beneficial markers such as immunoglobins to protect the host against invasion of pathogens into its body. • Upregulated antioxidant biomarkers by promoting synthesis of mRNA, which produces enzymes that combat oxidative stress.	• Enhanced T-SOD, GSH-Px, CAT, and glutathione transferase activities, while reducing the malondialdehyde content. • Reduced the H:L ratio in chickens, indicating successful stress mitigation. • Improved ovarian immune function by improving the expression of the ovarian tissue (toll-like receptor-4) while reducing pro-inflammatory cytokines (mRNA levels). • Improved bird immune response by modulating the regulatory T cells, which help reduce severe feather pecking and plumage damage: enhanced bird health and welfare.	Both probiotics and nucleotides have been shown to reduce the H:L ratio and pro-inflammatory biomarkers, using them together could boost overall bird health. Probiotics restore the balance of gut microbiota, while nucleotides act as immunomodulators by providing materials required for the replication of immune cells. The restoration of beneficial gut microbiota coupled with better overall immune responses should boost gut health and related outcomes in birds.
Gut function	• Increases the relative population of beneficial microbiota in the gut, mainly Lactobacillus. • Enhance the epithelia barrier function by proliferating the concentration of enterocytes, mucosal protein, DNA, and increasing villi height. • Prevent transcellular pathogenic invasion by attaching and binding to the villi. • Upregulate the production of immunoglobins, which bind antigens and hinders them from passing through the intestinal mucosal membrane	• Reduced diamine oxidase activity and D-lactate level by modulating the gastrointestinal tract. • Improved intestinal structure by enhancing the villus height and reduces crypt depth by modulating the host intestinal microbial environment. • Increases the population of beneficial caecal microbiota such as *Synergistota, Bacteroidota*, and *Firmicutes* in the gut.	Probiotics increase height of villi and maintain the integrity of the intestinal mucosa. They also restore the balance of the gut microbiota in stressed birds. Nucleotides, on the other hand, enhance the structural integrity of the gut and provide protection to the gut microbiome by initiating cellular repair of the gut, thereby reducing intestinal permeability and enhancing nutrient absorption. These are complementary actions whose combined effect should be positive for intestinal integrity and digestive efficiency.
External egg quality	• Increased strength of the eggshell and reduced proportion of damaged eggs by increasing the utilization and absorption of calcium in the GIT	• Enhanced eggshell thickness, strength, and eggshell percentage by increasing the blood serum calcium absorption and retention levels.	Both probiotics and nucleotides enhance strength and thickness of eggshells by promoting absorption and retention of calcium in gastrointestinal tract. Both additives optimize gut health, leading to improved calcium absorption. In addition, nucleotides can complement probiotics by facilitating the efficient transportation and retention of calcium in the egg.
Internal egg quality	• Enhanced albumen height and Haugh units. • Improved nutrient digestion and egg formation. • Enhanced bio-accessibility of nutrients and increases the concentration of carotenoids absorbed and deposited in the egg yolk. • Increased relative population of Bacteroidetes compared to Proteobacteria.	• Enhanced albumen parameters such albumen height, Haugh unit, and thick-to-thin ratio by increasing nutrient digestion and absorption through development of healthier intestinal structure.	Probiotics enhance nutrient absorption and create a healthier intestinal structure, while nucleotides deposit bioactive substances and increase the bio-accessibility of nutrients for egg formation. Together, probiotics and nucleotides could better enhance albumen height, Haugh units, and thick-to-thin ratio of eggs.
Egg functional properties	• Reduced egg pH through the vertical transfer mechanism. • Preserved albumen viscosity of the eggs by converting sugars to gluconic acid and prevents escape of CO_2 from the eggshell. • Reduced emulsion stability of the eggs	• Reduced egg yolk pH through the deposition of antioxidants, which then delay peroxidation. • Lower TBARS by improving the antioxidant status of the bird.	Probiotics and nucleotides reduce egg pH. Probiotics accomplish this by depositing antioxidants in the egg yolk, while nucleotides convert sugars into gluconic acid in the gastrointestinal tract. These two actions are complementary.

as well as accelerating tissue growth and repair (Wu et al., 2018; Xu et al., 2003; Liu et al., 2020; Mohamed et al., 2020; Kamel et al., 2021), which would maintain gut integrity and function. Higher feed intake leads to higher egg production and improved external egg quality due to enhanced nutrient sequestration that results in stronger eggshells (Elkaiaty et al., 2019; Attia et al., 2022). A robust eggshell reduces the likelihood of pathogens invading the egg yolk, thereby improving yolk fertility and reducing microbial load (Xu et al., 2003; Elkaiaty et al., 2019; Qiu et al., 2024). In addition, both nucleotides and probiotics have been shown to improve internal egg quality by enhancing albumen height, Haugh units, and the thick-to-thin albumen ratio (Obianwuna et al., 2022; Liu et al., 2021; Qiu et al., 2024). Nucleotides deposit bioactive substances and increase nutrient bioavailability while probiotics enhance nutrient absorption by promoting a balanced gut microbial structure (Tapingkae et al., 2018). Regarding external egg quality, probiotics and nucleotides strengthen the eggshell by improving calcium absorption and retention in the gastrointestinal tract (Tapingkae et al., 2018; Elkaiaty et al., 2019). Probiotics optimize gut health, leading to enhanced calcium absorption, while nucleotides facilitate the efficient transport and retention of calcium in the eggshell (Elkaiaty et al., 2019; Attia et al., 2022). Additionally, probiotics and nucleotides can combine to reduce egg pH (Nahariah et al., 2024).

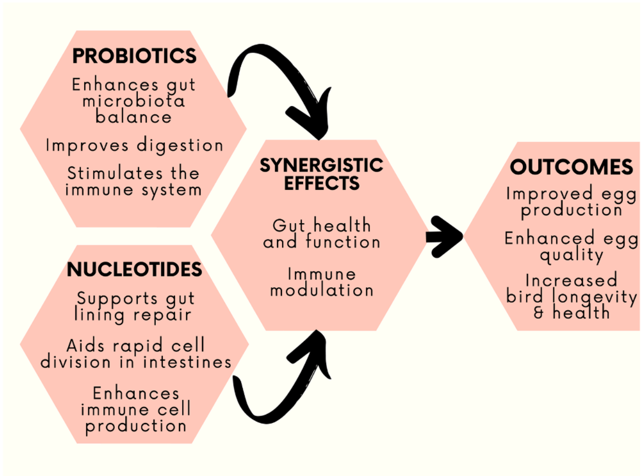

Fig. 2. Potential synergistic interaction between nucleotides and probiotics in layer chickens – A conceptual illustration of how their complementary mechanisms of action could enhance gut health, immune response, and overall performance in layer chickens.

Probiotics achieve this by depositing antioxidants in the egg yolk (Carvalho et al., 2022) while nucleotides convert sugars into gluconic acid in the gut, transferring it to the egg via vertical transfer mechanisms (Tapingkae et al., 2018). These actions can complement each other resulting in greater effects that are also consistent.

Potential limitations for nucleotide-probiotic blends

Although the use of nucleotides and probiotics has been shown to improve bird health and product quality in chicken layers, these additives may present certain limitations when combined. For instance, most studies on nucleotide supplementation have focused on yeast-based nucleotides, which contain additional compounds like amino acids. These compounds may directly contribute to improved bird health and product quality, making it challenging to attribute these benefits solely to nucleotides. Therefore, it is essential to evaluate the effectiveness of pure nucleotides (with >80 % nucleotide content) as mitigators of stress-induced maladies in intensively reared poultry. However, pure nucleotides are costly and have competing industrial applications, which can limit their availability and use in poultry production. On the other hand, the efficacy of both nucleotides and probiotics is influenced by several factors, including the composition of the gastrointestinal tract (GIT) microbiota, storage conditions, and methods of administration. Inconsistent outcomes for both additives have been reported, often due to variations in bird genetics, diet, environmental conditions, and potential interactions with other feed components. When combining probiotics and nucleotides, there is potential for antagonistic effects, such as competition for absorption in the gut. Understanding these interactions is essential for optimizing their synergistic use. Additionally, the effective dosages for both probiotics and nucleotides must be carefully determined through dose-response studies to identify the most beneficial combinations and avoid any adverse interactions.

Conclusion and future research prospects

This review highlights the potential of probiotics and nucleotides as alternatives to antibiotic growth promoters in poultry, particularly layer chickens. Both additives independently enhance gut health, boost immunity, and improve performance, though outcomes are often inconsistent. Exploring their combined use offers a promising solution, leveraging complementary mechanisms: probiotics modulate gut microbiota, while nucleotides support cellular growth and repair. Together, they could improve nutrient absorption, feed efficiency, and gut health more consistently than when used alone, thus optimizing bird performance, egg production, and quality. To fully realize this potential, detailed research is needed to clarify the biochemical and physiological pathways behind their synergistic effects. Future studies should explore a range of probiotic strains and nucleotides from various sources and purities. Dose-response experiments are essential to determine the optimal inclusion levels for both additives, as the ideal combination will depend on these factors. The selection of probiotics and nucleotides for testing will be guided by their availability, cost, and other practical considerations. Research should encompass different stages of poultry development, from in ovo to adult laying hens, to identify the most effective timing for administering these additives to achieve maximum benefit. In these studies, biomarkers such as intestinal and pancreatic digestive enzymes, histomorphological development, intestinal permeability, gut microbiome composition, intestinal gene expression, and effective immunity should be measured to assess gut health and immune status. Other proposed biomarkers for gut health include mucin-2, TLR-4, occludin, serum fluorescein isothiocyanate dextran (FITC-d), and ovotransferrin (Mantzios et al., 2024). For immune function, biomarkers such as interferon gamma (INF-γ), insulin-like growth factor (IGF), interleukins (IL-1β, IL-2, IL-6, IL-10), mucin (MUC2), and TTF2 (Transcription Termination Factor 2) have been used in chickens (Wu et al., 2018; Mohamed et al., 2020; Kamel et al., 2021; Rady et al., 2023; Ismail et al., 2024). Inflammatory markers like interleukin-17 (IL-17A), interleukin-22 (IL-22), and interferon (IFN-α) have also been used to assess immune function (Wu et al., 2018; Mohamed et al., 2020; Kamel et al., 2021; Rady et al., 2023; Ismail et al., 2024).

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used AI-assisted technologies to improve readability and language use in some parts of the paper. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Declaring of competing interest

All authors have no competing interests to declare.

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References

Agyare, C., Boamah, V.E., Zumbi, C.N., Osei, F.B., 2018. Antibiotic use in poultry production and its effects on bacterial resistance. Antimicrobial Resistance—A Global Threat. IntechOpen, pp. 33–51. https://doi.org/10.5772/intechopen.79371.
Ahire, J.J., Jakkamsetty, C., Kashikar, M.S., Lakshmi, S.G., Madempudi, R.S., 2021. *In vitro* evaluation of probiotic properties of *Lactobacillus plantarum* UBLP40 isolated from traditional indigenous fermented food. Probiotics Antimicrob. Proteins 13, 1413–1424.
Andam, K.S., Arndt, C.., Hartley, F., 2017. Eggs before chickens?: assessing Africa's livestock revolution with an example from Ghana. Intl. Food Policy Res. Inst. 1687.
Aro, S., Arogbodo, J., Ahmed, M., Ademola, O., 2021. Effects of stocking density on the performance characteristics, egg quality, and nutrient composition of the eggs of Japanese quails (*Coturnix coturnix japonica*). J. Sci. Res. Med. Biol. Sci. 2, 11–22.
Attia, Y.A., Al-Khalaifah, H., Abd El-Hamid, H.S., Al-Harthi, M.A., Alyileili, S.R., El-Shafey, A.A., 2022. Antioxidant status, blood constituents and immune response of broiler chickens fed two types of diets with or without different concentrations of active yeast. Animals 12, 453.
Ayana, G.U., Kamutambuko, R., 2024. Probiotics in disease management for sustainable poultry production. API 2024, 4326438.

Aziz, A.A.A., Aziz, E.S.A.A., Khairy, M.H., Fadel, C., Giorgi, M., Abdelaziz, A.S., 2024. The effect of butyric acid and nucleotides supplementation on broiler (Gallus gallus domesticus) growth performance, immune status, intestinal histology, and serum parameters. Open Vet. J. 14, 324.
Baines, D., Brown, M., 2016. Flavor enhancers: characteristics and uses. In: Caballero, B., Finglas, P.M., Toldrá, F. (Eds.), Encyclopedia of Food and Health. Academic Press, Oxford.
Balevi, T.U., Ucan, U..S., Coşun, B., Kurtoğu, V., Cetingül, I.S., 2001. Effect of dietary probiotic on performance and humoral immune response in layer hens. Br. Poult. Sci. 42, 456–461.
Barrett, N.W.K., Rowland, C.J., Schmidt, S.J., Lamont, M.F., Rothschild, C.M., Ashwell, C., Persia, M.E., 2019. Eff ects of acute and chronic heat stress on the performance, egg quality, body temperature, and blood gas parameters of laying hens. Poult. Sci. 98, 6684–6692.
Bhattacharyya, A., Chattopadhyay, R., Mitra, S., Crowe, S.E., 2014. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev. 94, 329–354.
Bindari, Y.R., Gerber, P.F., 2022. Centennial Review: factors affecting the chicken gastrointestinal microbial composition and their association with gut health and productive performance. Poult. Sci. 101, 101612.
Borda-Molina, Iffland, D.H., Schmid, M., Müller, R., Schad, S., Seifert, J., Tetens, J., Bessei, W., Bennewitz, J., A Camarinha-Silva, A., 2021. Gut microbial composition and predicted functions are not associated with feather pecking and antagonistic behavior in laying hens. Life 11, 235.
Carrey, E.A., Perrett, D.., Simmonds, H.A., 2013. Nucleic acids, purine, and pyrimidine nucleotides and nucleosides: physiology, toxicology, and dietary sources. In: Caballero, B. (Ed.), Encyclopedia of Human Nutrition, Third Edition. Academic Press, Waltham.
Carvalho, C...L.., Andretta, I., Galli, G.M., Stefanello, T.B., Camargo, N.D.O.T., Marchiori, M., Melchior, R., Kipper, M., 2022. Effects of dietary probiotic supplementation on egg quality during storage. Poultry 1, 180–192.
Cervantes, H.M., 2015. Antibiotic-free poultry production: is it sustainable? J. Appl. Poult. Res. 24, 91–97.
Castellini, C., Boggia, A., Cortina, C., Dal Bosco, A., Paolotti, L., Novelli, E., Mugnai, C., 2012. A multicriteria approach for measuring the sustainability of different poultry production systems. J. Clean. Prod. 37, 192–201.
Chichlowski, M., Croom, J., McBride, B.W., Havenstein, G.B., Koci, M.D., 2007. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: a brief review of current knowledge. Int. J. Poult. Sci. 6, 694–704.
Chowdhury, S.D., Ray, B..C., Khatun, A., Redoy, M.R.A., Afsana, A.S., 2020. Application of probiotics in commercial layer diets: a review. BJAS 49, 1–12.
Dancey, C.P., Attree, E..A., Brown, K.F., 2006. Nucleotide supplementation: a randomised double-blind placebo controlled trial of IntestAidIB in people with Irritable Bowel Syndrome [ISRCTN67764449]. Nutr. J. 5, 16.
De Cloet, C.A., Maina, A..N., Schulze, H., Bédécarrats, G.Y., Kiarie, E.G., 2023. Egg production, egg quality, organ weight, bone ash, and plasma metabolites in 30-week-old Lohmann LSL lite hens fed corn and soybean meal-based diets supplemented with enzymatically treated yeast. Poult. Sci. 102, 102527.
Deng, W., Dong, X.F., Tong, J.M., Zhang, Q., 2012. The probiotic Bacillus licheniformis ameliorates heat stress-induced impairment of egg production, gut morphology, and intestinal mucosal immunity in laying hens1. Poult. Sci. 91, 575–582.
Diarra, M.S., Malouin, F., 2014. Antibiotics in Canadian poultry productions and anticipated alternatives. Front. Microbiol. 5, 282.
Ding, T., Song, G., Liu, X., Xu, M., Li, Y., 2021. Nucleotides as optimal candidates for essential nutrients in living organisms: a review. J. Funct. Foods 82, 104498.
Dipeolu, M.A., Eruvbetine, D.., Oguntona, E.B., Bankole, O.O., Sowunmi, K.S., 2005. Comparison of effects of antibiotics and enzyme inclusion in diets of laying birds. Arch. Zootec Arch. Zootec. 54, 3–11.
Dumonceaux, T.J., Hill, J..E., Hemmingsen, S.M., Van Kessel, A.G., 2006. Characterization of intestinal microbiota and response to dietary virginiamycin supplem.Entation in the broiler chicken. Appl. Environ. Microbiol 72, 2815–2823.
Elkaiaty, A.M., Badran, A..M., Bayoumi, A., Eshera, A.A., A, A., El-Sayed, O., 2019. Effect of dietary yeast supplementation on productive performance, eggshell quality and lipid profile of laying hens. Egypt. Poult. Sci. J. 39, 567–578.
Ellakany, H.F., Abu El-Azm, I..M., Bekhit, A.A., Shehawy, M.M., 2008. Studies on the effects of enrofloxacin overdose on different health parameters in broiler chickens. J. Vet. Med. Res. 18, 176–186.
Engberg, R.M., Hedemann, M..S., Leser, T.D., Jensen, B.B., 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79, 1311–1319.
Fan, J., Zhang, Y., Zhou, H., Liu, Y., Cao, Y., Dou, X., Fu, X., Deng, J., Tan, B., 2023. Dietary malondialdehyde damage to the growth performance and digestive function of hybrid grouper (Epinephelus fuscoguttatus♀× E. lanceolatu♂). Animals 13, 3145.
Fantinato, V., Camargo, H.R., Sousa, A.L.O.P.D., 2019. Probiotics study with *Streptococcus salivarius* and its ability to produce bacteriocins and adherence to KB cells. Rev. Odontol. UNESP 48, e20190029.
Gadde, U., Kim, W.H., Oh, S.T., Lillehoj, H.S., 2017. Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim. Health Res. Rev. 18, 26–45.
Gautron, J., Réhault-Godbert, S., Van de Braak, T., Dunn, I., 2021. What are the challenges facing the table egg industry in the next decades and what can be done to address them? Animal 15, 100282.
Ghoname, M., Elnaggar, A., Hassan, S., Habashy, W., 2022. Effect of acute heat stress on production performance and egg quality in four strains of chickens. S. Afr. J. Anim. Sci. 52, 168–176.
Gou, H.Z., Zhang, Y..L., Ren, L.F., Li, Z.J., Zhang, L., 2022. How do intestinal probiotics restore the intestinal barrier? Front. Microbiol. 13, 929346.
Harper, G.C., Makatouni, A., 2002. Consumer perception of organic food production and farm animal welfare. Br. Food J. 104, 287–299.
Hartcher, K.M., Jones, B., 2017. The welfare of layer hens in cage and cage-free housing systems. WPS 73, 767–782.
Hassanein, I.I., Metwally, A.E., Abd Elbaky, H.E.M., M, H.E., 2023. Effects of dietary nucleotides supplementation on the performance of broiler chicks. Slovenian Vet. Res. 60.
He, S., Arowolo, M., Medrano, R., Li, S., Yu, Q., Chen, J., He, J., 2018. Impact of heat stress and nutritional interventions on poultry production. WPS 74, 647–664.
Hedlund, L., Jensen, P., 2022. Effects of stress during commercial hatching on growth, egg production and feather pecking in laying hens. PLoS One 17, e0262307.
Hu, W., He, Z., Du, L., Zhang, L., Li, J., Ma, Y., Bi, S., 2023. Biomarkers of oxidative stress in broiler chickens attacked by lipopolysaccharide: a systematic review and meta-analysis. Ecotoxicol. Environ. Saf. 266, 115606.
Iannetti, L., Romagnoli, S., Cotturone, G., Podaliri Vulpiani, M., 2021. Animal welfare assessment in antibiotic-free and conventional broiler chicken. Animals 11, 2822.
Ismail, R., Shousha S, S., Azab, R., 2024. Ameliorative effect of dietary nucleotides supplementation on antioxidative status and molecular expression of growth and immune related genes in broiler chickens. J. Adv. Vet. Res. J. 14, 567–571.
Jankowski, J., Tykałowski, B., Stępniowska, A., Konieczka, P., Koncicki, A., Matusevĭčius, P., Ognik, K., 2022. Immune parameters in chickens treated with antibiotics and probiotics during early life. Animals 12, 1133.
Glick, B., 1994. The bursa of Fabricius: the evolution of a discovery. Poult. Sci. 73, 979–983.
Jansen, L.J., Berentsen, R.J., Arends, M., Berendsen, B.J., 2020. The vertical transmission of antibiotic residues from parent hens to broilers. Food Addit. Contam. 37, 783–792.
Jiang, S., Hu, J.Y., Cheng, H.W., 2022. The impact of probiotic Bacillus subtilis on injurious behavior in laying hens. Animals 12, 870.
Kamel, N.F., Hady, M..M., Ragaa, N.M., Mohamed, F.nF., 2021. Effect of nucleotides on growth performance, gut health, and some immunological parameters of broiler chicken exposed to high stocking density. Livest. Sci. 253, 104703.
Karavolias, J., Salois, M.J., Baker, K.T., Watkins, K., 2018. Raised without antibiotics: impact on animal welfare and implications for food policy. Transl. Anim. Sci. 2, 337–348.
Khalifeh, M.S., Amawi, M.M., Abu-Basha, E.A., Yonis, I.B., 2009. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci. 88, 2118–2124.
Khan, R.U., Naz, S., 2013. The applications of probiotics in poultry production. WPS 69, 621–632.
Khan, S., Moore, R.J., Stanley, D., Chousalkar, K.K., 2020. The gut microbiota of laying hens and its manipulation with prebiotics and probiotics to enhance gut health and food safety. Appl. Environ. Microbiol. 86, e00600–e00620.
Kreuz, B.S., Rocha, G.C., Campos, P.H.R.F., Fonseca e Silva, F., Hannas, M.I., Albino, M.I. L.F.T., Borges, S.O., Calderano, A.A., 2020. Effects of dietary nucleotide supplementation on growth performance and physiology of broiler chickens under pre-and post-inflammatory challenge. RBZ 49, e20200117.
Kruger D., and Werf M.. 2018. Benefits of nucleotide supplementation in poultry. Ohly Germany, Hamburg.
Latif, A., Shehzad, A.S., Niazi, A., Zahid, W., Ashraf, M.W., Iqbal, A., Rehman, T., Riaz, R. M., Aadil, I.M., Khan, F., Özogul, J.M., Rocha, T., Estatbeyoglu, S.A., Korma, S.A., 2023. Probiotics: mechanism of action, health benefits and their application in food industries. Front. Microbiol. 14, 1216674.
Lawrence, R.A., 2022. 4 - Biochemistry of Human milk. In: Lawrence, R.A., Lawrence, R. M. (Eds.), Breastfeeding, Ninth Ed. Elsevier, Philadelphia.
Leung, H., Yitbarek, A., Snyder, R., Patterson, R., Barta, J.R., Karrow, N., Kiarie, E., 2019. Responses of broiler chickens to Eimeria challenge when fed a nucleotide-rich yeast extract1. Poult. Sci. 98, 1622–1633.
Li, D., Tong, Q., Shi, Z., Zheng, W., Wang, Y., Li, B., Yan, G., 2020. Effects of cold stress and ammonia concentration on productive performance and egg quality traits of laying hens. Animals 10, 2252.
Li, X.Y., He, C., Zhu, Y., Lu, N.H., 2020. Role of gut microbiota on intestinal barrier function in acute pancreatitis. World J. Gastroenterol. 26, 2187.
Li, G..M., Liu, L.P., Yin, B., Liu, Y.Y., Dong, W.W., Gong, S., Zhang J, J., Tan, J.H., 2020. Heat stress decreases egg production of laying hens by inducing apoptosis of follicular cells via activating the FasL/Fas and TNF-α systems. Poult. Sci. 99, 6084–6093.
Lima, E., Oliveira, M.B., Freitas, A., 2023. Antibiotics in intensive egg production: food Safety tools to ensure regulatory compliance. Food Chem. Adv. 3, 100548.
Liu, Y., Cheng, X., Zhen, W., Zeng, D., Qu, L., Wang, Z., Ning, Z., 2021. Yeast culture improves egg quality and reproductive performance of aged breeder layers by regulating gut microbes. Front. Micobiol. 12, 633276.
Liu, Q., Yu, Z., Tian, F., Zhao, J., Zhang, H., Zhai, Q., Chen, W., 2020. Surface components and metabolites of probiotics for regulation of intestinal epithelial barrier. Vet. Sci. 19, 1–11.
Ma, X., Chen, L., Yin, L., Li, Y., Yang, X., Yang, Z., Li, G., Shan, H., 2022. Risk analysis of 24 residual antibiotics in poultry eggs in Shandong, China (2018–2020). Vet. Sci. 9, 126.
Maldonado, J., Navarro, J., Narbona, E., Gil, A., 2001. The influence of dietary nucleotides on humoral and cell immunity in the neonate and lactating infant. Early Hum. Dev. 65, S69–S74.
Mantzios, T., Kiousi, D.E., Brellou, G.D., Papadopoulos, G.A., Economou, V., Vasilogianni, M., Kanari, E., Petridou, E., Giannenas, I., Tellez-Isaias, G., Pappa, A.,

2024. Investigation of potential gut health biomarkers in broiler chicks challenged by Campylobacter jejuni and submitted to a continuous water disinfection program. Pathogens 13, 356.
Mashaly, M., Hendricks, G., Kalama, M., Gehad, A., Abbas, A., Patterson, P., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poult. Sci. 83, 889–894.
Mateo, C.D., Stein, H.H., 2004. Nucleotides and young animal health: can we enhance intestinal tract development and immune function? Nutritional biotechnology in the feed and food industries In: Lyons, T.P., Jacques, K.A. (Eds.), Proceedings of Alltech's 20th Annual Symposium. Nottingham University Press, Nottingham, UK, pp. 159–168.
Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S.K., Côté, C., Ramirez, A.A., Godbout, S., 2018. Use of antibiotics in broiler production: global impacts and alternatives. Anim. Nutr. 4, 170–178.
Meseret, S., 2016. A review of poultry welfare in conventional production system. LRRD 28, 234.
Miles, R.D., Butcher, G..D., Henry, P.R., Littell, R.C., 2006. Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. Poult. Sci. 5, 476–485.
Mindus, C., van Staaveren, N., Bharwani, A., Fuchs, D., Gostner, J.M., Kjaer, J.B., Kunze, W., Mian, M.F., Shoveller, A.K., Forsythe, P., Harlander-Matauschek, A., 2021. Ingestion of *Lactobacillus rhamnosus* modulates chronic stress-induced feather pecking in chickens. Sci. Rep. 11, 17119.
Mohamed, F.F., Hady, M..M., Kamel, N.F., Ragaa, N.M., 2020. The impact of exogenous dietary nucleotides in ameliorating Clostridium perfringens infection and improving intestinal barriers gene expression in broiler chicken. VAS 10, 100130.
Nahariah, N., Hikmah, H., Yuliati, F., Murpiningrum, E., Jamaluddin, A., Kusuma, T., Febyana, A., Kartina, K., 2024. Application of saccharomyces cerevisiae in egg yolk to the functional and physicochemical characteristics of fermented eggs. Adv. Anim. Vet. Sci. 12, 125–131.
Nishiyama, T., Nakagawa, K., Imabayashi, T., Iwatani, S., Yamamoto, N., Tsushima, N., 2021. Probiotic *Bacillus subtilis* C-3102 improves eggshell quality after forced molting in aged laying hens. JPS 58, 230–237.
Obianwuna, U.E., Qiu, K., Chang, X.Y., Zhang, H.J., Wang, J., Qi, G.H., Sun, T.H., Su, Y. B., Wu, S.G., 2022. Enhancing egg production and quality by the supplementation of probiotic strains (*Clostridium* and *Brevibacillus*) via improved amino acid digestibility, intestinal health, immune response, and antioxidant activity. Front. Microbiol. 13, 987241.
Odenwald, M.A., Turner, J.R., 2017. The intestinal epithelial barrier: a therapeutic target? Nat. Rev. Gastroenterol. Hepatol. 14, 9–21.
Oelschlaeger, T.A., 2010. Mechanisms of probiotic actions–a review. Int. J. Med. Microbiol. 300, 57–62.
Onrust, L., Ducatelle, R., Van Driessche, K., De Maesschalck, C., Vermeulen, K., Haesebrouck, F., Eeckhaut, V., Van Immerseel, F., 2015. Steering endogenous butyrate production in the intestinal tract of broilers as a tool to improve gut health. Front. Vet. Sci. 2, 75.
Owusu-Doubreh, B.W.O.A., Abe-Inge, V., 2023. Antibiotic residues in poultry eggs and its implications on public health: a review. Sci. Afr. 19, e01456.
Qiu, Q., Zhan, Z., Zhou, Y., Zhang, W., Gu, L., Wang, W., He, J., Liang, Y., Zhou, W., Li, Y., 2024. Effects of yeast culture on laying performance, antioxidant properties, intestinal morphology, and intestinal flora of laying hens. Antioxidants 13, 779.
Pan, X., Cai, Y., Kong, L., Xiao, C., Zhu Q, Q., Song, Z., 2022. Probiotic effects of *Bacillus licheniformis* DSM5749 on growth performance and intestinal microecological balance of laying hens. Front. Nutr. 9, 868093.
Park, J.H., Sureshkumar, S., Kim, I.H., 2020. Egg production, egg quality, nutrient digestibility, and excreta microflora of laying hens fed with a diet containing brewer's yeast hydrolysate. J. Appl. Anim. Res. 48, 492–498.
Patterson, J.A., Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82, 627–631. https://doi.org/10.1093/ps/82.4.627.
Petruzziello, C., Saviano, A., Ojetti, V., 2023. Probiotics, the immune response and acute appendicitis: a review. Vaccines 11, 1170.
Phillips, I., Casewell, M.T., Cox, B., De Groot, C., Friis, R., Jones, C., Nightingle, R., Preston, R., Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53, 28–52.
Popova, T., 2017. Effect of probiotics in poultry for improving meat quality. Curr. Opin. Food Sci. 1472–1477.
Preller, M., Manstein, D.J., 2017. Myosin Motors: structural aspects and functionality☆. In: Giordano, A., Ranganathan, S., Cotter, P.D., Vitale, I. (Eds.), Reference Module in Life Sciences. Elsevier, pp. 72–75.
Qiu, Q., Zhan, Z., Zhou, Y., Zhang, W., Gu, L., Wang, Q., He, J., Liang, Y., Zhou, W., Li, Y., 2024. Effects of yeast culture on laying performance, antioxidant properties, intestinal morphology, and intestinal flora of laying hens. Antioxidants 13, 779.
Rady, W.F., Sayed, A.B.M., Abdel-Raheem, H.A., 2023. Effect of dietary supplementation of echinacea and nucleotides on productive performance, intestinal histomorphology and gene expression of broiler chickens. Assiut Vet. Med. J. 69, 141–155.
Ravindran, V., 2013. Alternative feedstuffs for use in poultry feed formulations. Poultry Development Review. FAO, pp. 72–75.
Ronquillo, M.G., A Hernandez, J.C., 2017. Antibiotic and synthetic growth promoters in animal diets: review of impact and analytical methods. Food Control 72, 255–267.
Rutz, F., Xavier, E.G., Anciuti, M., Roll, V.F.B., Rossi, P., 2007. The role of nucleotides in improving broiler prestarter diets: the Brazilian experience. Nutritional biotechnology in the feed and food industries:. In: Proceedings of Alltech's 23rd Annual Symposium. The New Energy Crisis: Food, Feed or Fuel?. Pelotas, Brazil, 2007. Universidade Federal de Pelotas, Brazil, pp. 175–181.
Sauer, N., Mosenthin, R., Bauer, E., 2011. The role of dietary nucleotides in single-stomached animals. Nutr. Res. Rev. 24, 46–59.
Servin, A.L., 2004. Antagonistic activities of *lactobacilli* and *bifidobacteria* against microbial pathogens. FEMS Microbiol. Rev. 28, 405–440.
Singh, M., Groves, P.J., 2020. Welfare implications for barn (and aviary) egg production systems. Anim. Prod.Sci. 61, 837–847.
Sjofjan, O., Adli, D.N., Harahap, R.P., Jayanegara, A., Utama, D.T., Seruni, A.P., 2021. The effects of lactic acid bacteria and yeasts as probiotics on the growth performance, relative organ weight, blood parameters. And immune responses of broiler: a meta-analysis. F1000Research 10, 1–19.
Stein, H.H., Kil, D.Y., 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. Anim. Biotechnol. 17, 217–231.
Svihus, B., 2014. Function of the digestive system. J. Appl. Poult. Res. 23, 306–314. https://doi.org/10.3382/japr.2014-00937.
Takiishi, T., Fenero, C.I.M., Câmara, N.O.S., 2017. Intestinal barrier and gut microbiota: shaping our immune responses throughout life. Tissue Barriers 5, e1373208.
Tang, D., Li, Z., Mahmood, T., Liu, D., Hu, Y., Guo, Y., 2020. The association between microbial community and ileal gene expression on intestinal wall thickness alterations in chickens. Poult. Sci. 99, 1847–1861.
Tapingkae, W., Panyachai, K., Yachai, M., Doan, H., 2018. Effects of dietary red yeast (Sporidiobolus pararoseus) on production performance and egg quality of laying hens. J. Anim. Physiol. Anim. Nutr. 102, e337–e344 (Berl).
Tarawali, S., Herrero, M., Descheemaeker, K., Grings, E., Blümmel, M., 2011. Pathways for sustainable development of mixed crop livestock systems: taking a livestock and pro-poor approach. Livest. Sci. 139, 11–21.
Tenrisanna, V., Rahman, M., 2023. Opportunities of free-range laying hens farming and economic advantages (A case study in Indonesia). In: Proceedings of the AIP Conference. AIP Publishing.
Thanh, N.T., Loh, T..C., Foo, H.L., Hair-Bejo, M., Azhar, B.K., 2009. Effects of feeding metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faecal microbial population, small intestine villus height and faecal volatile fatty acids in broilers. Br. Poult. Sci. 50, 298–306.
Thema, K., Mlambo, V., Snyman, N., Mnisi, C.M., 2019. Evaluating alternatives to zinc-bacitracin antibiotic growth promoter in broilers: physiological and meat quality responses. Animals 9, 1160.
Valini, G.A.C., Duarte, M...S., Calderano, A.A., Teixeira, L.M., Rodrigues, G.A., Fernandes, K.M., Veroneze, R., Serão, N.V.L., Mantovani, H.C., Rocha, G.C., 2021. Dietary nucleotide supplementation as an alternative to in-feed antibiotics in weaned piglets. Animal 5, 100021.
Velazquez-Meza, M...E.., Galarde-López, M., Carrillo-Quiróz, B., Alpuche-Aranda, C.M., 2022. Antimicrobial resistance: one health approach. Vet. World 15, 743.
Wang, J., Yao, L., Su, J., Fan, R., Zheng J, J., Han, Y., 2023. Effects of Lactobacillus plantarum and its fermentation products on growth performance, immune function, intestinal pH, and cecal microorganisms of Lingnan yellow chicken. Poul. Sci. 102, 102610.
Wang, Y., Wang, B., Zhan, X., Wang, Y., Li, W., 2022. Effects of glucose oxidase and its combination with B. Amyloliquefaciens SC06 on intestinal microbiota, immune response and antioxidative capacity in broilers. Animal 16, 100473.
Wu, G., Gunawardana, P., Bryant, M.M., Roland, D.A., 2008. Influence of dietary energy and antibiotic on performance, egg solids, and egg quality in Bovans White and Dekalb White hens. JAPR 17, 323–330.
Wu, C., Yang, Z., Song, C., Liang, C., Li, H., Chen, W., Lin, W., Xie, Q., 2018. Effects of dietary yeast nucleotides supplementation on intestinal barrier function, intestinal microbiota, and humoral immunity in specific pathogen-free chickens. Poult. Sci. 97, 3837–3846.
Xu, H., Lu, Y., Li, D., Yan, C., Jiang, Y., Hu, Z., Zhang, Z., Du, R., Zhao, X., Zhang, Y., Tian, Y., 2023. Probiotic mediated intestinal microbiota and improved performance, egg quality and ovarian immune function of laying hens at different laying stage. Front. Microbiol. 14, 1041072.
Xu, Z.R., Hu, C..H., Xia, M.S., Zhan, X.A., Wang, M.Q., 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82, 1030–1036.
Yang, Y., Iji, P.A., Choct, M., 2009. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. WPSl 65, 97–114.
Yazhini, P., Visha, P., Selvaraj, P., Vasanthakumar, P., Chandran, V., 2018. Dietary encapsulated probiotic effect on broiler serum biochemical parameters. Vet. World 11, 1344–1348.
Yin, S., Cui, H.X., Peng, J., Fang, Z., Zuo, J., Deng, X., Wang, B., Wu, B., Guo, H., 2015. Toxic effect of $NiCl_2$ on development of the bursa of Fabricius in broiler chickens. Oncotarget 7, 125.
Yitbarek, M.B., Berhane, G., 2014. Livestock production systems analysis. Am. Int. J. Contemp. Sci. Res. 1, 16–51.
Zaheer, K., 2015. An updated review on chicken eggs: production, consumption, management aspects and nutritional benefits to human health. Food Nutr. Sci. 6, 1208.
Zhan, H.Q., Dong, X..Y., Li, L.L., Zheng, Y.X., Gong, Y.J., Zou, X.T., 2019. Effects of dietary supplementation with *Clostridium butyricum* on laying performance, egg quality, serum parameters, and cecal microflora of laying hens in the late phase of production. Poult. Sci. 98, 896–903.
Zhao, X., Zhou, J., Liang, W., Sheng, Q., Lu, L., Chen, T., Chen, J., Tan, K., Lv, Z., 2021. Probiotics mixture reinforces barrier function to ameliorate necrotizing enterocolitis by regulating PXR-JNK pathway. Cell Biosci. 11, 1–14.
Zhen, W., Liu, Y., Shao, Y., Ma, Y., Wu, Y., Guo, F., Abbas, W., Guo, Y., Wang, Z., 2021. Yeast β-glucan altered intestinal microbiome and metabolome in older hens. Front. Microbiol. 12, 766878.