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PHYTOBIOTICS AS POTENTIAL REGULATORS OF THE GUT MICROBIOME COMPOSITION AND FUNCTIONAL ACTIVITY IN BROILER CHICKENS — A MINI-REVIEW

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Abstract

The efficiency of raising broiler chickens directly depends on the functional state of the gastrointestinal tract (N. Abdelli et al., 2021). The gut microbiome plays a key role in modulating the immune system and regulating digestive function. The relationship between diet and taxonomic profile is of particular interest to functional foods that have a positive effect on the microbiome (S. Khan et al., 2020). Metabolites synthesized by intestinal microorganisms serve as the main modulators of cross-communication between the host and the microbiome. Among these are short-chain fatty acids, tryptamine, conjugated linoleic acids, indole and its derivatives, as well as bile acids (S.A. Lee et al., 2017; S. Khan et al., 2020). Consequently, the microbiome is a fundamental link in maintaining productive interactions between the host and the intestine (S.A. Lee et al., 2017). Phytobiotics (FB) serve as a safe and effective alternative to feed antibiotics (M. Kikusato et al., 2021). The purpose of this review is to systematize information on the effectiveness of FB as potential regulators of the intestinal microbiome of broiler chickens. The beneficial functions of plant extracts mainly depend on their specific bioactive components (organic acids, polysaccharides, flavones), which can be synthesized as antimicrobial agents against pathogenic microorganisms (O.A. Bagno et al., 2018; J.J. Flees et al., 2021). It is known that the mechanism of action of FB consists in the destruction of the membrane of pathogenic microorganisms, modification of the cell surface with a change in virulence, stimulation of the immune system (S. Diaz-Sanchez et al., 2015). The contact of the microbiome and phytochemicals is a two-way process in which bacteria metabolize polyphenols into simpler metabolites, in turn, polyphenols affect the population of intestinal microorganisms, leading to a shift in metabolic activity (Y. Iqbal et al., 2020). FB control the growth and taxonomic composition of the intestinal microbiome, since phytochemicals, like prebiotics, have a positive effect on the state of the gastrointestinal tract even with minimal absorption in the small intestine (J. Martel et al., 2020). The feeding of phytochemicals is associated with high productivity indicators. It was found that the addition of the FB diet has a positive effect on the state of the metabolic activity of the organism and an increase in its adaptive potential, which is caused by the activation of the expression of certain genes (*IL6* and *BPIFB3*) in both infected and uninfected birds (G.Y. Laptev et al., 2021). Plant compounds can not only directly improve the health of broiler chickens, but also modulate the microbiota of the gastrointestinal tract and enhance the stimulating effect of productivity (O.A. Bagno et al., 2018). A review of the studies conducted on this topic demonstrates contradictory results. Therefore, studies of the dynamics of a multifactorial relationship between the environment, the host and the gut microbiome will allow for elucidating in-deep mechanisms of FB action on the intestinal ecosystem in broilers.

Keywords: phytobiotics, phytogenic compounds, broiler chickens, productivity, growth stimulants, microbiome

To meet the growing market demand for poultry meat, producers are forced to grow broiler chickens in conditions that provide the fastest possible increase in body weight, and therefore there is a need to use various growth promoters. However, consumers increasingly prefer natural products, which leads to a restriction of the use of feed antibiotics. From 2018 to 2023, the global phytobiotics (PhB) market is projected to expand from approx. US$ 631.4 million to over US$ 962.5 million [1].

The productivity of broiler chickens is directly related to the functional state of the intestine, which is determined by the structure of the diet and the activity of the gastrointestinal tract (GIT), intestinal microbiome and the immune system associated with it [2]. The gut microbiome plays a key role in modulating the immune system, digesting nutrients, and regulating GIT function. Such effects are mediated by complex microbial interactions, qualitative and quantitative characteristics of metabolites produced by members of the microbial community or resulted from nutrient transformation [3]. The microbiota provides intestinal homeostasis, forms tolerance to infections and non-pathogenic stressors [4]. Metabolites synthesized by gut microorganisms serve as the main modulators of cross communication between the host and the microbiome. These include short-chain fatty acids, tryptamine, conjugated linoleic acids, indole and its derivatives, and bile acids [3]. Therefore, the microbiome is a fundamental link in the interaction between the intestine and other body systems of the bird, which ensures its high productivity [5]. Since broiler chickens are not tolerant to potentially pathogenic microorganisms such as *Escherichia coli*, *Salmonella* and *Clostridium perfringens*, the functional state of the gastrointestinal tract requires special attention [6].

Establishing a relationship between diet and the taxonomic profile of the gut microbiota has increased interest in functional foods that have a positive effect on the gut microbial community and, as a result, overall host health. One such product is PhB as a safe and effective alternative to feed antibiotics [1]. PhBs are mainly known for their transcription-modulating action and pronounced antioxidant, anti-inflammatory, and antimicrobial activity [7). Plant secondary metabolites have been shown to have properties comparable to antibiotic growth promoters that help maintain gut health and improve overall performance in broiler chickens [6).

PhBs have a stimulating effect due to chemical properties and palatability (i.e., PhBs change feed attractiveness and intake), antimicrobial activity, improved digestion and absorption of nutrients with enhanced intestinal functions, as well as direct and indirect anabolic effects on target tissues through endocrine and antioxidant defense systems [8]. The variable composition of PhB makes it difficult to establish the possible mechanisms of their effect on the body of broilers, in particular, on the ecosystem of the digestive tract [6]. It is the impact on the microbiome that mediates the impact on productivity, and, accordingly, on the efficiency of growing broilers [9].

The purpose of this review is to systematize information on the effectiveness of PhBs as potential regulators of the taxonomic composition and activity of the intestinal microbiome in broiler chickens.

Physicochemical properties of phytobiotics. Phytogenic additives affect the state of the gastrointestinal tract, which is primarily reflected in productivity indicators [7, 10].

PhBs are a heterogeneous group of plant-derived feed additives with a high content of bioactive compounds [7]. The raw materials for them are plant extracts or parts of plants (leaves, rhizomes, roots, flowers or bark, bulbs, stems, as well as fruits and seeds) with a maximum accumulation of biologically active substances [11]. Laf extracts have a higher biological activity. This is due to the fact that the composition of the leaves is characterized by an abundance of pharmacologically active components, in particular polyphenols [12].

Based on their chemical structure, PhBs are divided into six categories: phenolic compounds, alkaloids, nitrogen-containing compounds, organosulfur compounds, phytosterols, and carotenoids [13]. In practice, bioactive extracts are also used, such as essential oils (EOs), pigments (mainly carotenoids, anthocyanins), alkaloids, glycosides, phenolic acids, phytosterols, flavonoids [11]. EOs are herbal essences obtained by distillation with water and/or steam (10), which are a mixture of chemical compounds — terpenes, terpenoids and polyphenols (phenylpropenes, flavonoids) [14]. EOs are valuable due to their antimicrobial, antiviral, antioxidant and antiparasitic properties [2]. The antioxidant activity of FB, especially phenolic acids and flavonoids, is determined mainly by the structure and delocalization of electrons above the aromatic nucleus [7].

One of the main advantages of PhBs is reduced or no toxicity, high availability, and ease of use in agricultural practice [15].

Mechanism of action of phytobiotics. It is assumed that PhBs have a direct and indirect effect on the microbiota of broiler chickens. The direct mechanism is to destroy the membrane of pathogenic microorganisms; cell surface modifications affecting virulence; stimulation of the immune system, in particular the activation of lymphocytes, macrophages and NK cells; protecting the intestinal mucosa from colonization by bacterial pathogens; encouraging the growth of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* [16].

The mechanism of the direct action of EOs has not been fully elucidated, however, there is evidence of a violation of the integrity of the cell membrane of microorganisms after interaction with EOs [17], which, due to their lipophilic characteristics, are able to diffuse through the lipid layer. Bacterial cell membranes, which provide a relatively stable internal environment for the life of bacteria, are responsible for the barrier and selective transport of metabolites, and also perform other important biological functions. i.e., maintaining hormonal homeostasis, enzymatic reactions (membrane proteins often have enzymatic activity and/or participate in its membrane regulation), cell recognition, and participation in signal transduction [18, 19]. For normal growth, bacteria need to maintain the morphological and functional integrity of the membrane. It is known that antimicrobial agents of various origins can influence cell metabolism, homeostasis, and morphology by targeting bacterial surface structures and modulating their permeability [20]. Membrane permeabilization leads to changes in ion influx, cytoplasmic coagulation, and structural damage to membrane-bound proteins, which determine antimicrobial effects [10, 21].

Oregano EO has pronounced antioxidant and antimicrobial properties, while the most bioactive compound in oregano EO is carvacrol. The action of carvacrol appears to be related to the hydroxyl group in its phenol ring and its hydrophobic nature; carvacrol interacts with the lipid bilayer of cytoplasmic membranes, disrupting their integrity and causing leakage of cellular contents - ions, adenosine triphosphate and nucleic acid [22].

There is a hypothesis that Gram-positive bacteria are more susceptible to the action of hydrophobic compounds such as EOs [23]. The reason for this is attributed to the presence of a cell wall in gram-positive bacteria, consisting of a thick layer of peptidoglycan, which is associated with hydrophobic proteins and teichoic acid, which facilitates the penetration of molecules that also have hydrophobic properties. Gram-negative bacteria have a more complex cell wall consisting of an outer membrane linked to an inner layer of peptidoglycan via lipoproteins. The outer membrane contains proteins and lipopolysaccharides (lipid A), which makes it more resistant to hydrophobic EOs [24]. Possible mechanisms of the effect of EO on bacterial reproduction are the destruction of the outer membrane or the double layer of phospholipids, changes in the composition of fatty acids, an increase in membrane fluidity with subsequent release of protons from the cell, impaired glucose uptake, and inhibition of enzyme activity [23].

The composition of EOs can include 100 chemical compounds, with the predominance of one or more of them forming the chemotype of EOs [25]. The biological function of essential oils is related to the activity of their main components, the structural configuration of the compounds present, their functional groups, and possible synergistic effects [26]. It has been established that combinations of EOs have a high antimicrobial effect due to the synergistic effect, when the combined effect of several compounds is greater than the sum of their effects separately [17].

The carbonyl groups of anethole and fenchone from fennel attach to cell membrane proteins and exert an antimicrobial effect by disrupting the structure of the microbial plasma membrane lipid layer, resulting in loss of cellular content [27].

The mediated mechanism of action of PhBs is due to their ability to modulate the functional activity of the microbiome, the change in which is associated with the state of the mucous membrane of the gastrointestinal tract (structure of microvilli, depth of crypts) [28]. It should be noted that the absorption of PhBs, including EO, in the small intestine is very low, approx. 2-15% [28, 29]. PhBs are mostly polyphenols. Due to low absorption, approx. 90% of phenolic compounds enter the colon unchanged [29]. More than 90% of the polyphenols entering broiler intestines are digested by representatives of the intestinal microbiota, but not digestive enzymes. Microbial degradation of polyphenols with the formation of intermediate products, including aglycones and aromatic acid metabolites, increases their bioavailability and enhances the effect of PhBs. PhBs modulate the microbiome to a greater extent in the caecum than in the ileum. This is due to the peculiarities of the composition of microbial associations, PhBs metabolism, and their absorption in different parts of the intestine [30].

The interaction of microbiota and phytochemicals is a two-way process. For example, bacteria degrade polyphenols, metabolizing them to simpler compounds, and polyphenols affect the composition of the population of intestinal microorganisms, leading to a shift in their metabolic activity [29]. PhBs are able to control the growth and composition of the gut microbiome, since phytochemicals, like prebiotics, have a positive effect on the state of the gastrointestinal tract even with minimal absorption in the small intestine [31].

Some PhBs lead to an increase in the production of short-chain fatty acids - lactate, acetate and butyrate, which indicates an increase in the metabolic breakdown of carbohydrates. Many PhBs themselves can be sources of organic acids and reduce the pH in the gastrointestinal tract, which inhibits the growth of pathogenic bacteria (particularly *Escherichia coli*) [32].

We noted above that morphological features (villus length, crypt depth and height) serve as indicators of intestinal health [29]. Elongation of the villi has a positive effect on digestion, improves the absorption of nutrients and promotes weight gain. Therefore, changes in gut morphology affect nutrient uptake and animal performance [29]. It has been shown that PhBs affect the functional state of the villi in the small intestine. An increase in villus size correlates with increased metabolite absorption. PhBs can lead to an increase in the height of the villi and the depth of the crypts in the intestines of broilers. In addition, one of the common effects of PhBs is a decrease in the abundance of E. coli, which also contributes to an increase in the surface area of the villi [33]. When the intestinal microbiota is modified by PhBs, the digestibility and absorption of nutrients increases [32].

Effect of phytobiotics on the microbiota of broiler chickens. Among PhBs, essential oils are more popular due to their pronounced antimicrobial properties (9). At the same time, each of the components that make up the essential oil has its own mechanism of action, which together leads to synergy [16]. The addition of EO to broiler diets improves growth performance, regulates gut microbiome composition (Table), and significantly reduces the effects of exposure to pathogens such as *Salmonella* [34], *E. coli* [35], *Clostridium perfringens* [36] and *Eimeria* spp. [37].

Phytobiotics and their effect on the taxonomic profile of different parts of the intestine in broiler chickens

Producer plant	Active ingredient	Effect	References
	Ileum		
Capsicum annum L.	Capsaicin	↓ *Escherichia coli*, *Enterobacteriaceae*, gram-positive lactobacilli	(9)
Foeniculum vulgare Miller)	Anethole, limonene, fenchon, estragole, safrole, α-pinene, camphene, betapinene, sabeinene, β-myrcene, phellandrene, cisocymene, paracymol, γ-terpinene, camphor	↓ *E. coli*; ↑ *Lactobacillus* spp.	(27)
Coriandrum sativum L.	Linalool, α-pinene, γ-terpinene, geranyl acetate, camphor, geraniol	↓ *E. coli*; ↑ *Lactobacilli*	(46)
Pimpinella anisum L.	Trans-anethole, eugenol, anisaldehyde, polyacetylenes, methylchavicol, scopoletin, estragole, coumarins, umbelliphron, estrols and polyenes	↓ Colonization by pathogenic bacteria	(47)
Trachyspermum ammi L.	γ-Terpinene, thymol, p-cymol, β-pinene	↓ *E. coli*; ↑ *Lactobacilli*	(48)
Magnolia officinalis Rehder & E.H. Wilson	Magnolol, honokiol	↓ *Streptococcus* and unidentified *Cyanobacteria*; ↑ α-Diversity и β-diversity, abundance of *Firmicutes*, *Lactobacillus*	(43)
Lavandula angustifolia Miller	Myrcene, α-pinene, caryophyllene, linalool, α-terpineol, borneol, camphor, carvone, eukarvone, linalo-ol acetate, lavandulilacetate, geranyl cetate, neral	↓ *E. coli* and coliform bacteria; ↑ Probiotic bacteria, bacteriostatic effect	(49)
Thymus vulgaris L.	Thymol, carvacrol	↓ *E. coli* and the total number of gram-negative bacteria; ↑ Abundance of lactic acid bacteria	(50)
Allium cepa L.	Propyl thiosulfinate, propyl propyl panthiosulfonate	↓ *E. coli*; ↑ *Lactobacillus* и *Streptococcus*	(33, 45)
	Cecum		
Rumex nervosus L.	Gallic acid, catechin, chlorogenic acid and caffeine	↓ *E. coli*	(51)
Castanea sativa Miller and *Schinopsis lorentzii*	Tannins	↓ *Bacteroides*; ↑ Members of *Clostridiales* families *Ruminococcaceae* and *Lachnospiraceae*	(52)
Oríganum vulgáre L.	Carvacrol, thymol	↓ *Streptococcus*; ↑ *Enterococcus*, *Lactobacillus*; ↑ Production of acetic and butyric acids	(22, 40)
Eucalyptus globulus La Billardière	1,8-cineole (eucalyptol), α-pinene, - phellandrene, γ-terpinene, α-terpineol, cymene, limonene, and spatulenol	↓ *E. coli*; ↑ Abundance of lactic acid bacteria	(44)
Cinnamomum zeylanicum Jan Svatopluk Presl	Коричный альдегид, эвгенол, 3-фенил, 2-пропеналь	↓ *E. coli*; ↑ *Lactobacillus* и *Bifidobacterium*	(53, 54)
Melissa officinalis L.	Gallic acid, catechin, chlorogenic acid, caffeic acid, ellagic acid, epicatechin,	↑ Number of colonies of enterococci	(55)
Allium hookeri Thwaites	Rutin and quercetin	↓ *Eubacterium nodatum*, *Marvinbryantia*, *Oscillospira* и *Gelria*	(12)
	Small intestine		
Nelumbo nucifera Gaertner	Alkaloids and flavonoids	↓*Peptostreptococcaceae*; ↑ *Clostridiaceae* and *Bacteroidales* S24-7	(20)
Allium sativum L.	Alliin, diallyl sulfides, allicin	↓ *Escherichia coli*; ↑ *Lactobacillus*	(56)
	Jejunum		
Curcuma longa L.	Curcumin	↓ *E. coli*; ↑ Total number of aerobic mesophilic and lactic acid bacteriaй	(57)

Continued Table

Ileum and caecum			
Camellia sinensis L.	Catechins	↓ *E. coli*, *Lactobacillus*	(58)
Duodenum, jejunum and ileum/caecum			
Macleaya cordata Willdenow	Sanguinarine, chelerythrine	↓ *Corynebacterium*, *Brachybacterium*, *Dietzia*, proteobacteria; ↑ *Lactobacillus*, *Ruminococcaceae*, *Lachnospiraceae*, *Clostridium*, *Butyricicoccus*, *Faecalibacterium*, *Firmicutes*, the ratio *Firmicutes/Bacteroidetes*	(35, 41)

N o t e. Symbols ↓ and ↑ mean a corresponding decrease and increase in the indicator.

Lactobacilli metabolize polyphenols, producing energy substrates for cells. *Lactobacillus acidophilus* is able to convert plant glycosides to form aglycones [38). *L. delbrueckii* and *Eubacterium ramulus* can use these aglycones as nutrient substrates [29]. *Bacteroides ovatus*, *Veillonella* sp. and *Ruminococcus productus* further metabolize aglycones via ring opening, lactone cleavage, decarboxylation, dehydroxylation, demethylation, reduction and isomerization [39].

It has been shown that the addition of 1% oregano powder to the diet of broiler chickens leads to a selective change in certain groups of bacteria. In particular, the representation of *Streptococcus* decreases, *Enterococcus* increases, the ratio of *Lactobacillus* species changes. The production of short-chain fatty acids was 61% higher in the experimental groups compared to the control. As a result, these effects led to improved morphology of the intestinal mucosa, stimulation of the immune system and, in general, an increase in the productivity of broilers [40]. The antimicrobial activity of extracts of various oregano species has been demonstrated on gram-negative bacteria, including Salmonella typhimurium, *E. coli*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, and *Enterobacter cloacae*, as well as on Gram-positive bacteria, *Staphylococcus epidermidis*, *Listeria monocytogenes*, and *Bacillus subtilis* [17].

PhBs of *Macleaya cordata* extract contains sanguinarine and chelerythrin. These substances are included in the group of benzylisoquinoline alkaloids with antimicrobial and anti-inflammatory properties. In addition, sanguinarine is very similar in molecular structure to the benzylisoquinoline alkaloid berberine, which has a high clinical efficacy in the treatment of a number of diseases due to the modulation of the intestinal microbiota [35, 41]. *Macleaya cordata* extract leads to an increase in the presence of *Lactobacillus* in the foregut. Moreover, due to the mechanism of nutrient cross-use, lactate produced by *Lactobacillus* can be metabolized by anaerobic bacteria to form butyrate. Butyrate serves as an energy source for intestinal cells, which is accompanied by a pronounced anti-inflammatory effect [41]. It has been established that when sanguinarine is added to the diet of broilers, the taxonomic profile of the microbiota shifts towards *Ruminococcaceae*, *Clostridium*, *Lachnospiraceae*, *Butyricicoccus*, and *Faecalibacterium*, which produce short-chain fatty acids [35].

Berberine as a natural pentacyclic isoquinoline alkaloid isolated from the roots, rhizomes, stems, bark and leaves of *Rhizoma coptidis*, Cortex phellodendri, *Hydrastis canadensis* and *Berberis* has a strong antimicrobial, antioxidant, anti-inflammatory and immunomodulatory effect. Feeding this alkaloid to broilers leads to changes in the composition of the microbiota, in particular, to a decrease in the representation of the phylum *Firmicutes* and the genera *Lachnospiraceae*, *Lachnoclostridium*, *Clostridiales*, and *Intestinimonas*, with a simultaneous increase in the abundance of the phylum *Bacteroidetes* and the genera *Bacteroides* and *Lactobacillus* [42].

Magnolol and its isomer honokiol are the main phenolic compounds extracted from the root and bark of *Magnolia officinalis*. Magnolol and honokiol have anti-inflammatory, antioxidant, antibacterial activity and are involved in the regulation of metabolism. The effect of these substances on the functional parameters of the microbiota of the ileum of broilers was established, in particular, an increase in the activity of metabolic pathways associated with the transformation of valine, aspartate, glutamate and dibasic acids was noted. In addition, the addition of magnolol enhanced the pathways associated with the biosynthesis of ansamycins. Rifamycins, which are part of ansamycins, exhibit antimicrobial activity against aerobic bacteria and *Salmonella* [43].

The constant feeding of citrus extract with a high content of flavone to broiler chickens leads to the modulation of the structure of the microbiota, in particular to an increase in the number of *Bifidobacterium* and activation of the expression of mRNA of the intestinal barrier genes (*ZO-1* and *Claudin*) in the ileum. Concomitantly, there was an increase in *Lactobacillus*, lactate, and short-chain fatty acids in the cecum, followed by a decrease in protein fermentation products (ammonia, p-cresol, indole, total amines, spermidine, methylamine, and tyramine) [32].

The antibacterial activity of eucalyptus (*Eucalyptus globulus*) is due to the biological action of 1,8-cineol, limonene and -pinene. It has been shown that the addition of eucalyptus EO to the diet helps to reduce the number of *E. coli* and increase the population of lactic acid bacteria in the contents of the caecum [44].

Broiler chickens are fed with *Allium onion* extracts as a feed additive. Onions contain organosulfur compounds. Among them, thiosulfinates have high biological activity. They are volatile and easily evaporate, which leads to a significant change in the final concentrations in the feed. Thiosulfinates in the presence of O_2 rapidly form highly stable thiosulfonates [45]. *Allium hookeri* contains allicin, which gives this onion its intense spicy flavor. Allicin is an organosulfur compound with characteristic antibacterial activity against various microorganisms, including *Staphylococcus* and *Pseudomonas*. This effect is due to a chemical reaction with thiol groups of enzymes that affect the metabolic activity of cysteine proteinases, putative factors of bacterial virulence [12].

As a source of PhBs, both whole plants and isolated active compounds (apigenin, quercetin, curcumin and resveratrol, terpenes eugenol, thymol, carvacrol, capsaicin, artemisinin and aldehydes cinnamaldehyde and vanillin) are studied. These molecules have not only antimicrobial (including antibacterial, antifungal, antiviral and antiprotozoal), but also anti-inflammatory, antioxidant, immunomodulatory properties and improve the morphology and functionality of the intestinal mucosa [59-61].

Thus, over the past 20 years, interest in the use of phytobiotics (PhBs) in animal husbandry has increased significantly. Phytobiotics are substances of plant origin with a high content of bioactive compounds, the main action of which is to modulate the intestinal microbiota and its metabolism. Due to potential bioactivity, PhBs may be used as the main agent in organic poultry farming. The beneficial functions of plant extracts depend on their specific components such as organic acids, polysaccharides and flavones, which can be synthesized as antimicrobial agents. It is important to understand the mechanism of action of each of the components, which, in turn, is determined by its physicochemical properties and mediates the effects of synergy or antagonism of these bioactive compounds. The available data confirm that the effectiveness of PhBs used as feed additives for broiler chickens is due not only to the modulation of the composition and metabolic activity of the symbiotic microflora in the gastrointestinal tract, but also to the improvement of its functional state even with minimal absorption of PhBs in the small intestine. A decrease in the proportion of pathogenic microorganisms in the intestines and an increase in the abundance of probiotics significantly increases the productivity of broiler chickens. The mechanism of action of PhBs is determined, in particular, by their lipophilic structure and the ability to act on the cytoplasmic cell membranes. The effect of PhBs is more pronounced in the caecum, but not in the ileum. It is obvious that the interactions in the microbiota—host—environment during the use of dietary PhBs are complex, especially considering the diversity of these chemical compounds, their antagonistic and synergistic relationships. Therefore, further studies will elucidate the mechanisms of PhB action on the gut ecosystem of broilers.

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