



## Revolutionising broiler nutrition: The role of probiotics, fermented products, and paraprobiotics in functional feeds

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### ARTICLE INFO

#### Keywords:

Broilers health  
Feed additives  
Fermented plant products  
Probiotics  
Paraprobiotics

### ABSTRACT

Advancements in nutrition, inheritance, housing management, and research on chicken well-being and welfare have driven growth in poultry production. However, the unregulated use of antibiotic growth promoters has led to antibiotic resistance and drug residues, prompting several nations, including European Union members, to ban antibiotics in animal feed. Researchers have focused on safe alternatives such as probiotics, fermented probiotics products, and paraprobiotics. Therefore, nowadays normal diet is supplemented with probiotics that act as an immunomodulator which effectively sustains the healthy microbiota, regulate different blood parameters and enhances meat quality. Moreover, the feeding of probiotics-fermented plant-based food with basal diet provides a double benefit to chicken health as it gets the benefits from probiotics as well as dietary polyphenols. Finally, the addition of paraprobiotics increases the overall gut immunity in the broiler chickens.

### 1. Introduction

By 2029, worldwide consumption of poultry meat is expected to reach approximately 145 Mt [1]. Numerous potential food ingredients can be generated as byproducts of poultry meat manufacturing. It is considered the most demanding sector of animal husbandry because of its rapid reproduction capacity, short breeding period, and comparatively lower investment costs than other livestock branches. This allows for faster turnover and efficient and cost-effective production [2–4]. Chicken meat is nutritionally favorable, with a high energy value and easy digestibility. It is widely accepted across different cultures and religions, making it accessible to diverse societies [5–11]. As an essential component of the global food chain, modern grill chicken production attempts to meet the strict laws governing the quality and safety of items intended for consumption. A high-quality and safe feed supply is crucial

for meeting production goals, increasing total productivity, and raising the standard of animal-derived meals [12,13]. However, in addition to essential nutrients, various dietary supplements enhance the benefits and minimize the adverse effects on chicken bodies [14,15].

Functional feed formulations have been developed to improve the nutritional value and health of farm animals. These formulations frequently include phytochemical feed additives (PFAs), a class of natural supplements [4,16]. PFAs are composed of plant extracts, medicinal plants, spices, and essential oils. The intricate composition of PFAs poses challenges for conducting systematic and comprehensive studies to assess their effectiveness and safety [17]. The variability in bioactive compounds within batches of PFAs can be attributed to factors, such as the botanical origin of the plant, growing locations, production methods, storage conditions, effective dosage, and environmental and rearing conditions. This complexity makes optimization a further

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arduous [17,18]. Furthermore, an inclusive range of phytochemicals, including essential oils (EOs), are highly volatile and reactive, causing rapid evaporation. This rapid evaporation contributed to significant fluctuations in the final feed additive. Yang et al. [17] highlighted that most phytochemicals require a higher minimum inhibitory concentration (MIC) than is economically feasible. Variations in concentration levels result in variable effectiveness in stimulating poultry growth, and can contribute to higher production costs.

Increasing the body weight of poultry is another method of increasing meat production. This approach has been used for nearly 150 years and involves the use of antibiotic growth promoters (AGPs) [19]. AGPs are antibiotics administered sub-therapeutically, such as streptomycin, tetracycline, or avoparcin, which result in increased body weight or a lower feed conversion ratio (FCR) [20]. Growth hormones are also used for this purpose. In 2006, the European Union (EU) implemented a significant decision through Council Regulation (EC) No 2821/98, which prohibited the use of antibiotics and hormonal growth promoters in livestock feed. Several countries, including New Zealand, South Korea, and Mexico, have followed the EU's strategy of banning AGPs. Although it is considered a reliable measure, many countries view this approach as radical. The USA, Australia, Japan, and Canada have partially banned antibiotic-derived additives while excluding certain ones [19]. In 2015, the rise of bacterial strains resistant to colistin, an antibiotic of last resort, posed a significant threat to community health. The presence of *mcr-1* can confer resistance to colistin, making the bacterial strain less susceptible to this antibiotic. The gene can be transferred between different bacteria through plasmids, small mobile pieces of DNA that can move between cells, and even between different species of bacteria; colistin usage was prohibited by the Chinese government on animals in 2016 [21]. In order to stop the spread of resistance and retain the effectiveness of colistin in research settings, the Chinese Ministry of Agriculture also outlawed the use of colistin as a feed additive for livestock in 2017 [22]. The Food Safety and Standards Authority of India (FSSAI) has also made ongoing efforts to prohibit colistin [23].

The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations jointly defined probiotics in 2002 as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." These are typically bacteria or yeasts that are similar to useful microorganisms naturally present in our bodies, especially in the digestive system. They can be consumed as dietary supplements or found in certain foods such as yogurt, kefir, and fermented vegetables [24]. An effective probiotic should possess certain characteristics, including being part of the intestinal microflora, resistance to the acidic gastric environment, easy adherence to the intestinal epithelium, and the ability to maintain a healthy balance of microflora in the intestines [25]. In poultry farms, probiotics are most commonly introduced through feed. Other methods such as gavages (vaccines or drops), sprays, coated capsules, granules, pills, tablets, and powder sachets are used [24]. Furthermore, in addition to incorporating probiotics into the feed, breeders are increasingly choosing to administer formulations through water [26,27]. Each approach follows a distinct path with the shared objective of enhancing the poultry output.

Canibe and Jensen [28] fed fermented goods to pigs for a long time owing to their beneficial effects on gut health and growth. Fermented feed is increasingly being incorporated into broiler rations to enhance productivity [29,30]. Fermented products are being explored for their health benefits and as cost-effective alternatives to expensive conventional feed ingredients, such as yellow corn, in broiler diets [31–34]. Several studies have been conducted in the past decade on incorporating inactivated or killed probiotics, or paraprobiotics, into the feed [35].

In this review, we explored the factors that influence chicken health and the prospective advantages of probiotics in this context. This study focuses on the immunomodulatory capabilities of probiotics, their role in sustaining a healthy gut microbiota, and their effects on blood

parameters, growth performance, and meat quality in chickens. Additionally, this review has discussed the relevance of commercially available probiotics, along with the introduction of the concept of fermented probiotic products and the use of paraprobiotics as functional feeds for broiler chickens.

## 2. Health factors affecting chickens

### 2.1. Use of antibiotics

Antibiotics are commonly used to prevent or treat illnesses in different sectors, such as agriculture, fish aquaculture, livestock feeding, and human or veterinary medicine [36]. They are generally effective against bacteria and fungi but not against viruses. The classification of antibiotics is based on three main factors: chemical structure, action method, and the specific microorganisms they target. Antibacterial drugs are classified into two main categories: bactericidal and bacteriostatic drugs. Bactericidal drugs disrupt bacterial cell walls. Bacteriostatic drugs inhibit bacterial growth during the stationary phase. They achieve this by targeting proteins involved in bacterial proliferation. Furthermore, these drugs facilitate phagocytosis of bacteria by the immune system. Since antibiotics have been widely used, animal mortality rates have decreased. A significant risk to antibiotic effectiveness is also associated with excessive and widespread antibiotic use [36]. Antibiotics are frequently used in poultry production, particularly in North America. The chicken industry has used antibiotics such as tetracycline, bacitracin, tylosin, salinomycin, virginiamycin, and bambarmycin for a variety of reasons [37]. Compared with the EU, tetracyclines make up only 37 % of the antimicrobials used in animals in the United States [38, 39]. The amount of antibiotics used in livestock and poultry can be estimated by examining the sales statistics of veterinary antimicrobial agents. These statistics provide insight into the extent of antibiotic use in these sectors. In 2015, 8361 tons of veterinary antimicrobial agents were sold in the EU, excluding growth promoters used in animal production [36,40]. In Canada, approximately 1500 tons of effective antimicrobial substances were provided for animal use in 2014, with a 5 % increase compared to the previous year. Less than 1 % of this sum was set aside for pets, with 99 % going to farms. The majority (81 %) of antimicrobials used in Canada's grill farms in 2014 were used to prevent coccidiosis and necrotic enteritis caused by *Clostridium perfringens*. Of this, 84 % of the antimicrobials were administered by incorporating them into the feed [36]. It is important to remember that nations participating in the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) program forbid the use of antibiotics as growth promoters [36]. This indicates a regulatory stance against the use of antibiotics to promote growth in livestock farming practices.

#### 2.1.1. Effects of antibiotics on the growth, digestive tract, and immune system of chickens

Several studies have suggested that the administration of subtherapeutic doses of antibiotics to poultry farm chickens not only aids in disease prevention, but also enhances feed conversion, increases growth rates, and ultimately improves meat production [41–46]. Broiler chickens are known to benefit from antibiotics by controlling gastrointestinal infections, altering intestinal microbiota, and modulating immune responses. However, the precise mechanisms underlying these effects remain unclear [20,47–49]. It is thought that antibiotics have an impact on the variety and relative number of bacteria in the chicken intestine. Antibiotics exert selection pressure on microbiota, which is likely to lead to the development of an ideal microbiota community [20]. Metagenome sequencing studies have shown that antibiotics such as salinomycin (60 ppm) and virginiamycin (100 ppm) in chickens can have a significant impact on the gut microbiome by altering its composition [36,50]. In broiler chickens, virginiamycin, used as a growth promoter, has been shown to promote the growth of *Lactobacillus species* in the gastrointestinal tract, particularly in the duodenum and

proximal ileum.

In contrast, when tylosin, a bacteriostatic antibiotic, is included in chicken feed, it causes a significant reduction in the population of *Lactobacillus* spp. in the ileum of chickens [51]. In several studies, *Lactobacilli* spp. consistently decreased following antibiotic treatment [48, 52, 53]. As primary commensal bacteria, *Lactobacillus* spp. play an important role in the synthesis of bile hydrolase salts [51]. When the population of *Lactobacillus* is reduced by antibiotic treatment, it can decrease the activity of bile hydrolase salts in the intestine. Consequently, conjugated bile salts become more abundant. Because conjugated bile salts promote lipid metabolism and energy harvesting, animals are likely to gain more weight [51]. The microbiota of chickens is crucial to both their immune system and overall health, and any changes in the dynamics of the microbiota can result in significant consequences. In addition to antibiotics, other factors can also influence the intestinal microbiome. The dynamics of the gut microbiome can be influenced by several variables, including chickens, living circumstances, microbial exposure, dietary patterns, and environment [48]. As part of examining and understanding the complex interplay between microbiota and chicken health, these factors should be considered alongside antibiotic use.

## 2.2. Dysbiosis

In dysbacteriosis or dysbiosis, the microbiota of the host organism undergoes detrimental changes, resulting in an imbalance between beneficial and harmful bacteria [54]. Maintaining a balanced gut microbiota is essential for preventing the colonization of pathogenic microorganisms, while preserving beneficial commensal bacteria [54]. Host organisms also carry opportunistic microbes that can proliferate and become pathogenic under favorable conditions. Broilers with dysbiosis exhibit intestinal inflammation and atrophic villi in the small intestine [55]. AGPs have been effective in preventing dysbiosis in chickens for several years. Dysbiosis has become a significant problem in poultry production as AGPs have been restricted [55, 56]. In addition to the absence of AGPs, dysbiosis can also be caused by dietary changes and the presence of mycotoxins, as well as infectious agents, such as *Clostridium perfringens* and *Coccidia* [57, 58]. As natural components of grains such as wheat, rye, and barley, non-starch polysaccharides (NSPs) can result in dysbiosis. Non-starch polysaccharides (NSPs) comprise various components, including cellulose, glucans, inulin, arabinoxylans, chitin, gums, and mucilage. These NSPs play a significant role in contributing to the dietary fiber content of food [59, 60]. Chicken enzymes do not easily digest insoluble NSPs but can be metabolized by the gut microbiome into short-chain fatty acids (SCFAs) [61]. Conversely, soluble NSPs have been observed to have adverse effects on villus surface area, height, width, and immunological response in the chicken gut. When present in the intestine, soluble NSPs tend to amplify the viscosity of gut digestion. This increased viscosity creates an anaerobic environment within the gut, providing suitable conditions for colonizing pathogenic anaerobic bacteria, such as *C. perfringens* and *Escherichia coli*. Consequently, these pathogenic microbes can induce acute inflammation [62–65].

Mycotoxins, which are harmful secondary metabolites generated by some fungi and molds, belong to genera such as *Aspergillus*, *Penicillium*, and *Fusarium* [66, 67]. In addition to their known antibacterial properties, mycotoxins can also induce dysbiosis through indirect effects on intestinal cells. These effects include intestinal epithelial cell apoptosis, increased gut porosity, enhanced mucus generation, harmful gut villi, and the disruption of compact junction proteins [68]. Dysbiosis results from alterations in the intestinal environment, which can negatively impact gut health and microbial balance. When present in feed, mycotoxins can increase the levels of pro-inflammatory cytokines and immunoglobulin A (IgA) in infected organisms [69]. The effects of mycotoxins are primarily observed in the gut of organisms, where the gut microbiome is responsible for the metabolism of mycotoxins into less toxic compounds [70]. The microbial biotransformation of mycotoxins

reduces their toxic effects on organisms [69]. An example of such detoxification is the microbiome transformation of aflatoxin, a common mycotoxin.

## 2.3. Heat stress

Heat (thermal) stress occurs when there is an imbalance between the body's thermogenesis (heat production) and dissipation (heat loss) [71]. The normal body temperature of chicken's ranges from 41 °C to 42 °C. To achieve optimal growth rates, chickens must be kept within the thermoneutral zone, typically at approximately 18-21 °C [72]. When ambient temperatures exceed the thermoneutral zone, they negatively affect poultry morphology, behavior, growth performance, and productivity [73–75]. Chickens respond naturally to thermal stress, where they tend to decrease their food consumption as a strategy to reduce thermogenesis; however, this reduced feed intake can harm their overall well-being [76]. Appetite has been identified as an important marker of thermal stress in chicken. Zhang et al. [77] conducted a study that illustrated the involvement of neuroendocrine signals, particularly leptin neuropeptide Y (NPY), in the regulation of hunger in chickens under thermoneutral conditions. Additionally, leptin has been found to impact the heat shock protein 70 (HSP70) gene expression in chickens. Under thermoneutral conditions, Figueiredo et al. [78] found that leptin suppresses HSP70 gene expression in chickens, highlighting the complex relationship between thermal stress, appetite regulation, and cellular stress. Moreover, thermal stress can affect fertility in male and female breeders differently. Bonato et al. [79] found that, while ambient temperature increases initially stimulate testicular growth and semen quantity in male breeders, further increases can suppress their reproductive capacity [80]. Consequently, sustained thermal stress may negatively affect the reproductive performance of male broiler chicken. A significant increase in the prevailing temperature over the thermoneutral zone can lead to elevated lipid peroxidation caused by the generation of reactive oxygen species. Oxidative stress can damage the testes and ultimately have detrimental effects on the semen quality in chickens. The effects of thermal stress on semen include reductions in semen volume, testicular weight, quantity and movement of sperm, and decreased spermatozoa viability. In addition, thermal stress may adversely affect the development and maturation of spermatids, spermatozoa, and spermatogonia [81]. Semen quality can be influenced by factors other than thermal stress, such as the pH and ion concentration. If these parameters are not optimized, poor quality spermatozoa can be produced; therefore, chickens may become infertile [81, 82].

Under heat-stress conditions, the immune system of chickens can be adversely affected. Prolonged exposure to high temperatures can lead to a condition called heat stress or exhaustion in chickens. Specific components of the immune system that can be affected include intra-epithelial lymphocytes and macrophages [73]. Heat induces oxidative damage to cell membranes, resulting in atrophy of lymphoid organs such as the thymus, bursa, and spleen, as well as a decrease in the volume of lymphoid follicles, medulla/cortex ratio, and lymphoid follicle volume [83–85]. It should be emphasized that heat stress has a negative impact on the functionality of the immune system, leading to an increased presence and proliferation of pathogenic bacteria in the crop and intestine. Furthermore, heat stress can cause morphological alterations in the intestinal lymphoid cells. The antigen-presenting system presents pathogens to immune cells, activating pro-inflammatory cytokines [86, 87]. In summary, heat stress in poultry adversely affects their immune response against invading pathogens.

## 3. Probiotic species and strains

Probiotics consist of microorganisms derived from seven genera: *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Saccharomyces*, *Propionibacteria*, *Streptococcus*, *Enterococcus*, and *Escherichia*. These microorganisms were identified based on specific strains, including the genus, species,

subspecies, and alphanumeric strain descriptions (Fig. 1) [96]. Many probiotic strains are obtained from fermented milk products such as kefir, kumiss, Maasai, or Kurut milk, which are significant sources of various lactic acid bacteria [102]. Lactic acid bacteria have also been isolated from breast milk and fermented foods, such as wine, beer, and soy products [96,103].

#### 4. Role of probiotics in chicken feeds

Probiotics can aid in preserving a healthy gut microbiome in chickens, which is essential for proper digestion, nutrient absorption, and immunological responses. (Fig. 2). In Table 1, an overview of different probiotics that have been utilized in chicken feed over the past six years is provided. The specific role of probiotics in chicken feed is discussed below.

##### 4.1. Immunomodulator

The best performance of chickens is dependent on a strong immune system, and there is a well-established link between the gastrointestinal tract and immune system. Numerous lymphoid cells, particularly intraepithelial lymphocytes, are present in the epithelial mucous membrane. Specialized lymphoid structures connected to the gastrointestinal lumen include Meckel's diverticulum, Fabricius bursa, cecal tonsils, and Peyer's patches [119,120]. This intricate network of lymphoid structures and cells plays a critical role in coordinating the actions of enteric neurons and gut immune cells in response to various stressors [121]. Study found that chickens fed with *Lactobacillus fermentum* and *Saccharomyces cerevisiae* showed higher proportions of T-lymphocytes expressing specific surface markers, such as the cluster of differentiation (CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup>), suggesting an immune-enhancing effect of these probiotic strains. In contrast, the administration of antibiotics has decreased the proportion of CD8<sup>+</sup> T lymphocytes in chickens [122]. In contrast, incorporating probiotics into poultry feed has been shown to increase the expression of anti-inflammatory cytokines, such as interleukin-2 (IL-2 and IL-10), while decreasing the levels of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 $\beta$ ), IL-6, interferon-gamma (IFN- $\gamma$ ), and tumor necrosis factor-alpha (TNF- $\alpha$ ) [98,110,123,124]. Furthermore, probiotics can boost macrophage activity, trigger both humoral and cellular immunological responses, and

increase antibody and cytokine synthesis and secretion [125]. Additionally, probiotics exert an influence on Toll-like receptors (TLRs), a type of pattern recognition receptor (PRRs) found in the gut epithelial cells responsible for recognizing various microbial compounds and triggering immune activation [126]. To maintain homeostasis in the host, TLRs serve as the initial signaling mechanism that activates immune responses against harmful gut microbes [127]. Oral administration of probiotics upregulates the mRNA expression levels of TLR2, TLR4, and TLR15 in both the jejunum and ileum of broiler chickens [128]. Furthermore, probiotics affect the expression of Myeloid Differentiation primary response gene 88 (MyD88), an adapter protein involved in downstream signaling pathways. Through the mediation of tumor necrosis factor receptor-associated factor 6 (TRAF 6) and TAK1-binding protein 1 (Table 1), this pathway promotes the innate immune response, ultimately leading to the activation of nuclear factor kappa B (NF- $\kappa$ B) [129]. Rajput et al. [128] demonstrated a significant increase in MyD88, TRAF6, Table 1, and NF- $\kappa$ B expression in the jejunum and ileum of chickens after probiotic administration.

##### 4.2. Maintaining a healthy microbiome in the gut

Lactate, propionate, acetate, and isovalerate are organic and SCFAs produced by probiotics. Consequently, the gut becomes acidic, making the growth of harmful microorganisms difficult [130]. Furthermore, *Lactobacillus* and *Bacillus* spp. generate bacteriocins that prevent the growth of various dangerous bacteria, including *Campylobacter*, *C. perfringens*, *E. coli*, *Staphylococcus aureus*, *Vibrio* spp., *Salmonella*, *Proteus*, and *Shigella* [131]. Furthermore, Izzuddin et al. [132] found that a liquid probiotic containing *Lactobacillus* sp., when used as a food additive, increased the number of lactic acid bacterial colonies in broiler chickens. Other studies have found that adding *Lactobacillus*, *Bacillus*, *Pediococcus*, and *Saccharomyces* to feed increases populations of good bacteria, such as *Lactobacillus* and *Bifidobacteria* while decreasing dangerous bacteria such as *E. coli* and total aerobes [133]. Additionally, supplementation of feed with 0.8 % dietary yeast culture has been shown to significantly increase the populations of *Ruminococcus*, *Propionibacterium clostridiales*, and *Bifidobacterium* while reducing the presence of *Enterobacteriaceae* species in broilers [134]. In a separate study conducted by Soumeih et al. [135], administering *Bacillus* spp. to mixed-sex ROSS308 broiler chickens resulted in an increase in the

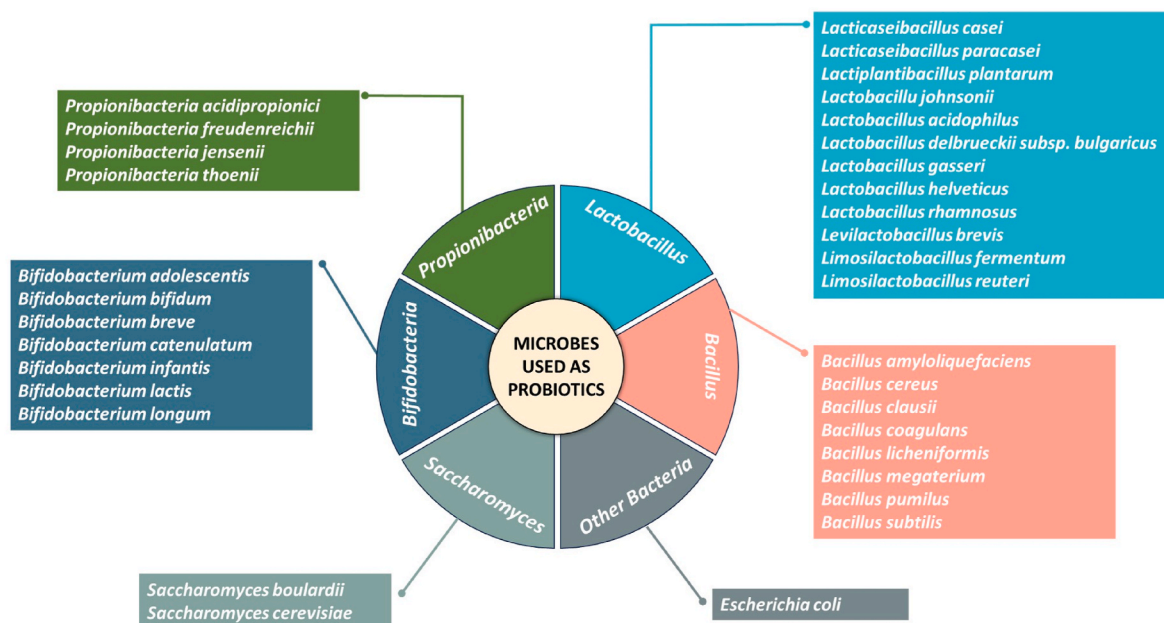


Fig. 1. Illustrative view of different types of microbial genera used as probiotics [88–101].

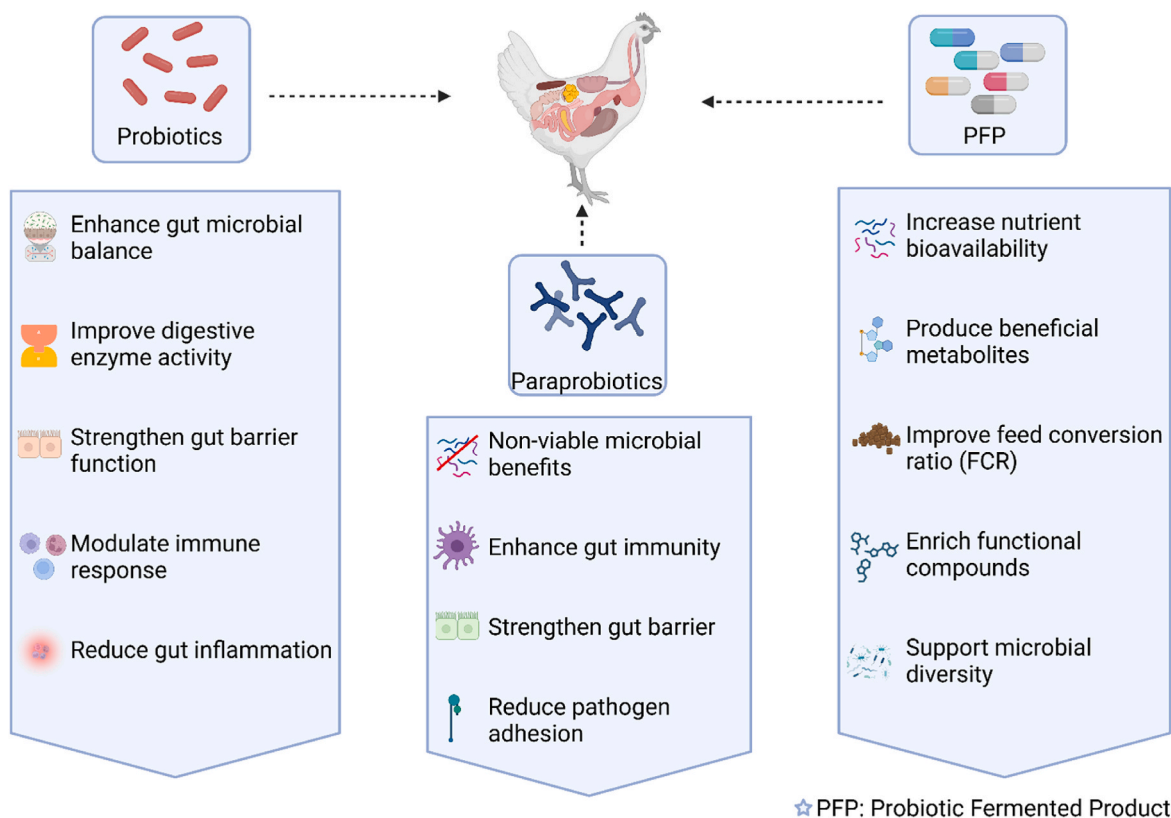


Fig. 2. A graphic illustration showing the role that probiotics, probiotic-fermented products, and paraprobiotics play in the gut of broiler chickens.

Firmicutes phylum, followed by Bacteroidetes. The five most abundant families were *Streptococcaceae*, *Lachnospiraceae*, *Peptostreptococcaceae*, *Ruminococcaceae*, and *Erysiphe-lotrichaceae*. Interestingly, the administration of *Bacillus* spp. also reduces the relative abundance of *Bacteroides fragilis* within the gut microbiota [135]. Yang et al. [136], reported that dietary supplementation of *Lactiplantibacillus plantarum* improves the health and growth of broilers, likely by shifting the gut microbiota toward bacteria that produces SCFAs. As a result, *L. plantarum* shows the potential of viable substitute to antibiotics in poultry breeding. Mirsalami and Mirsalami [137] observed that the incorporation of 0.5 % of both *Enterococcus faecium* and *Streptococcus thermophilus* in poultry feed enhances the relative abundance of *Ruminococcaceae* and *Faecalibacterium* which aid in effective management of diarrhoea and reduced presence of *Escherichia coli* in contrast to the control diet. In addition to that, supplementation of probiotics in the basal diet decreases the concentration of ammonia (NH<sub>3</sub>), carbon dioxide (CO<sub>2</sub>), hydrogen sulphide (H<sub>2</sub>S), and methane (CH<sub>4</sub>).

#### 4.3. Regulator of the blood parameters

Poultry farmers prioritize productivity while safeguarding the health and well-being of their birds and assuring the safety of their products for human consumption. Various methods have been investigated to accomplish this, including genetic engineering, alternative feed ingredients, and feed essences [138]. Blood tests are frequently used to assess the health and metabolic status of birds without harming them. Blood biochemical profiles, which include factors such as protein, albumin, glucose levels, alkaline phosphatase, electrolyte balance, total aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol, triglycerides, creatinine, and uric acid, can be analyzed to provide valuable diagnostic information about a variety of illnesses affecting the liver, kidneys, bones, and muscles [138,139]. This will allow researchers and industry professionals to monitor the health of birds throughout the experiment, without sacrificing them.

Supplementation with specific *Lactobacillus* spp strains *L. salivarius* strains isolated from chicken intestines and *L. casei* WB315 from the feedstuff of broiler chickens have shown significant benefits. It has been observed that such supplementation leads to a reduction in the serum levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglycerides in broiler chickens while also improving their body weight gain (BWG) [140,141]. Similarly, supplementation with other probiotic microorganisms such as *Enterococcus faecium* and *Bifidobacterium* spp. has also demonstrated a significant reduction in cholesterol and triglyceride levels in the sera of broiler chickens [142]. On the other hand, incorporating dietary yeast supplements, including commercially available yeast diets such as Microguard® (*Saccharomyces boulardii*), has been associated with decreased alkaline phosphatase and ALT activities, as well as lower plasma gamma-glutamyl transpeptidase and cholesterol concentrations [100,143]. These findings suggest that supplementing broiler chickens with specific probiotic strains and dietary yeast may have a positive effect on lipid metabolism and overall health.

#### 4.4. Growth performance and meat quality

The poultry meat production sector is expected to experience significant growth in countries such as Brazil, China, and the United States, as well as in the EU, particularly in Hungary, Poland, and Romania, where production costs are low [144]. Poland, for instance, saw an increase in poultry product exports, reaching 1.8 million tons in 2021, a 0.4 % rise compared to the previous year [144]. Consumers are increasingly looking for poultry raised on local and regional farms that place a high priority on the welfare of their animals [145]. An important factor that influences consumers' decisions to consume poultry meat is its caloric and nutritional value, as well as its potential health benefits. These factors are largely influenced by the feed provided to the chickens [144]. Therefore, it is important to inform consumers about the amount of nutrients and calories in uncooked meat from broiler chickens fed with substitute feed additives in order to influence their purchasing

**Table 1**  
Country-specific studies on the role of different probiotics in chicken feed between 2018 and 2025 (last 8 years).

| Country of study         | Verity of chicken/age/health status  | Type of probiotics   | Dose concentration   | Mode of administration | Health consequences   | References |
|--------------------------|--|--|--|------------------------|---|------------|
| United States of America | Cobb 500/1day/challenged with <i>Salmonella enteritidis</i>  | <i>Bacillus subtilis</i> CE330, <i>Enterococcus durans</i> CH33  | 0.5 g/kg of feed   | Oral (basal diet)      | Probiotic supplementation reduced the load in <i>Salmonella</i> by 38 % in comparison to the control; probiotic supplementation also did not change the weight gain in body, feed conversion ratio, intestinal histomorphology ( $p > 0.05$ ), or IL-10 and IL-1 $\beta$ gene expression  | [104]      |
| China                    | Arbor Acres/1day/healthy   | <i>Bacillus subtilis</i> strains DSM32324 and DSM32325   | 300 mg/kg, and 500 mg/kg   | Oral (basal diet)      | <i>B. subtilis</i> supplementation at 500 mg/kg and 300 mg/kg significantly increased the height of jejunal villus by 24.8 and 17.89 %, respectively and decreased the depth of jejunal crypt by 31.9 and 27.2 %, respectively after 21 days; <i>B. subtilis</i> supplementation also enhanced immunoglobulins and cytokine levels in both jejunal mucosa and serum; <i>B. subtilis</i> improved the butyrate-producing bacteria ( <i>Lachnospiraceae</i> , <i>Anaerostipes</i> , <i>Tyzzerella</i> , <i>Clostridium sensu stricto</i> 13, <i>Lachnospiraceae</i> UCG-010, and <i>Prevotellaceae</i> NK3B31_group) abundance and potential probiotics ( <i>Sutterella</i> ) abundance | [105]      |
| China                    | Cobb 500/1day/healthy  | <i>Bacillus subtilis</i>   | 500 g/ton of feed  | Oral (basal diet)      | Significant ( $p < 0.05$ ) improvement in the growth performance in birds taking probiotic-supplemented diet (PSD) was observed throughout the trial, along with increase in crypt depth and villus length as compared to other groups, and decreased <i>Clostridium perfringens</i> , <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> cecal populations in contrast to the control ( $p < 0.05$ )   | [106]      |
| India                    | Vencobb 430Y/1day/healthy  | <i>Lactococcus lactis</i> subsp. <i>lactis</i> BIONCL17752   | $4.0 \times 10^9$ cfu/kg   | Oral (basal diet)      | Treatment with probiotics resulted in reduction of <i>Firmicutes</i> and increase in <i>Bacteroidetes</i> (F/B ratio) in the gut of broilers, thus highlighting the beneficial effects of probiotic dietary supplementation; the group fed with probiotics exhibited higher carbohydrate-active enzymes (CAZyme) abundance including glycoside hydrolases (GH), carbohydrate-binding module (CBM) hydrolases, and glycoside transferases (GT), essential for degrading complex sugar molecules  | [107]      |
| China                    | Yellow-feathered chicks/1day/challenged with <i>Salmonella Typhimurium</i>   | <i>Lactiplantibacillus plantarum</i> GX17  | $1.5 \times 10^9$ cfu/chick  | Oral (Water)           | Probiotic medication could help ease weight loss after infection, decrease the count of bacteria in the liver, cecum, spleen, and reduce liver pathological damage amongst all treated participants; the commercial and <i>L. plantarum</i> probiotics could protect against <i>S. typhimurium</i> invasion by affecting the populations of <i>Anaerotruncus</i> , <i>Lactobacillus</i> , and <i>Colidextribacter</i> bacteria  | [108]      |
| China                    | Arbor Acres/1day/challenged with <i>Clostridium perfringens</i>  | <i>Lactobacillus acidophilus</i>   | $4.0 \times 10^6$ cfu/kg   | Oral (basal diet)      | Increased the body weight of broiler; reduced the intestinal lesion score of challenged broilers, the mRNA expression of pro-inflammatory cytokines, ileal populations of <i>Escherichia</i> and serum endotoxin content; increased the intestinal <i>Lactobacillus</i> populations   | [109]      |
| China                    | Cobb 500/1day/challenged with <i>Eimeria maxima</i> and <i>Eimeria acervulina</i>  | <i>Lactobacillus johnsonii</i> BS15  | $1 \times 10^6$ cfu/g  | Oral (basal diet)      | Might be contributed to inhibit liver inflammation by alleviating the effect of pro-inflammatory pathways including Toll-like receptor (TLR) signalling pathway, CD80 and IL-1 $\beta$ genes; in addition, this TLR-targeting BS15 prevention may effectively reduce damages due to subclinical necrotic enteritis (SNE) infection  | [110]      |
| Japan                    | Chunky broiler/1day/challenged with heat-killed <i>Salmonella Typhimurium</i>  | <i>Lactobacillus reuteri</i>   | $1 \times 10^8$ cfu  | Oral (water)           | Enhanced intestinal mucosal physical barrier function, but not the expression of other immune-related factors   | [111]      |
| Brazil                   | Cobb/1day/challenged with ([new wood shavings used as litter, low stocking density (8 birds/m <sup>2</sup> ) and daily-cleaned bell drinkers] called low | <i>Lactobacillus acidophilus</i> , <i>Bacillus subtilis</i> , <i>Bifidobacterium bifidum</i> and <i>Enterococcus faecium</i> | $1 \times 10^9$ cfu/g 2.8 $\times 10^9$ cfu/g, $2 \times 10^9$ cfu/g $2 \times 10^9$ cfu/g | Oral (basal diet)      | Probiotic does not promote satisfactory improvements, regardless of the environmental challenge   | [112]      |

(continued on next page)

Table 1 (continued)

| Country of study | Verity of chicken/age/health status  | Type of probiotics  | Dose concentration       | Mode of administration      | Health consequences  | References |
|------------------|--|---|--------------------------|-----------------------------|--|------------|
| China            | challenge) and higher challenge (re-used (after three broods of broilers) wood shavings as litter, high bird-density (16 birds/m <sup>2</sup> ) and bell drinkers cleaned every two days)<br>Yellow-feather broilers/100days/healthy | <i>Lactobacillus plantarum</i> and <i>Bacillus subtilis</i>                                   | $1.5 \times 10^9$ cfu/mL | Oral (basal and water)      | <i>L. plantarum</i> could increase the intestinal enzyme activity of broilers, while decreasing the plasma malondialdehyde (MDA) content, increasing the albumin (ALB) and total protein (TP) content, decreasing the plasma aspartate transferase (AST) content, increasing the concentrations of plasma IgA and CD8 molecules, improving the jejunum structure; <i>B. subtilis</i> increased the concentration of plasma AST and CD8 molecules of broilers. Additionally, <i>B. subtilis</i> also increases the plasma glutathione peroxidase (GSH-Px) content, but not affecting the plasma biochemical indexes   | [113]      |
| Vietnam          | Noi/1day/challenged with <i>Salmonella</i> Typhimurium   | <i>Lactobacillus plantarum</i> and <i>Bacillus subtilis</i>                                   | $10^7$ - $10^8$ cfu/g    | Oral (basal diet)           | Lower mortality rate and improved body weight gain but did not affect carcass characteristics  | [114]      |
| China            | Arbor Acres/1day/healthy   | <i>Lactobacillus casei</i> , <i>L. acidophilus</i> , and <i>Bifidobacterium</i>               | $5 \times 10^9$ cfu/g    | Oral (water)                | Supplementation of 1 % probiotics increased in growth performance, carcass traits, immune function, gut microbial population, and antioxidant capacity   | [115]      |
| Poland           | Ross308/1day/healthy   | <i>Lactobacillus plantarum</i> KKKP 593/p and <i>Lactobacillus rhamnosus</i> KKP 825          | $1 \times 10^9$ cfu/g    | Oral (basal diet and water) | Addition of bacteria to water had a favorable effect on higher live body weight of chickens during the first (starter) and the second (grower) period of rearing compared to the control group and the group receiving bacteria in feed; total feed intake and feed conversion ratio was higher in the groups receiving bacteria than in the control group; mortality among chickens receiving lactic acid bacteria (LAB) was significantly reduced, wherein the lowest mortality was observed among chickens receiving bacteria in feed; feeding chickens with potentially probiotic bacteria had an impact on inhibition the growth of <i>Clostridium perfringens</i> in the intestine and did not change biochemical and haematological parameters of blood and results of slaughter analysis compared to the control group | [116]      |
| Iran             | Cobb 500/1day/healthy  | <i>Bacillus subtilis</i> JQ61816  | NS                       | Oral (basal diet)           | Up-regulated the mucin 2 gene expression   | [117]      |
| Iran             | Ross 308/1day/healthy  | <i>Bacillus subtilis</i> and <i>Bacillus licheniformis</i>                                    | $1 \times 10^9$ cfu/g    | Oral (basal diet)           | <i>B. licheniformis</i> improved broilers' body weight gain (BWG), feed conversion ratio (FCR), and production efficiency factor (PEF); adding <i>B. licheniformis</i> caused the lowest feed cost per kilogram weight gain and the highest return of investment (ROI); probiotic treatments significantly decreased ileal pH of the broilers; probiotic treatments improved apparent metabolizable energy (AME) and total tract digestibility of protein; <i>B. licheniformis</i> was superior to <i>B. subtilis</i> in improving broiler chickens' BWG (2580.70 vs. 2427.45 g) and their PEF (418.95 vs. 374.49)   | [118]      |
| Germany          | Cobb 500/4-6weeks/healthy  | <i>Bacillus subtilis</i> DSM 32315 (BS), and <i>Bacillus amyloliquefaciens</i> CECT 5940 (BA) | NS                       | Co-cultured                 | <i>Bacillus</i> strains enhanced T cell activation and proliferation   | [97]       |
| China            | White-feathered broilers/6days/challenged with <i>Salmonella</i> Enteritidis (SE)  | <i>Bacillus pumilus</i> TS1   | $1.4 \times 10^7$ cfu/mL | Oral (basal diet)           | <i>Bacillus pumilus</i> TS1 alleviated oxidative stress damage caused by SE and attenuated the inflammatory response in broilers through MAPK/PKC regulation of HSPs/HIF-1 $\alpha$  | [98]       |

CD8: Cluster of differentiation 8, CD80: Cluster of differentiation 80, IL-10: Interleukin-10, IL-1 $\beta$ : Interleukin-1 beta, NS: Not specified, CFU: Colony-forming unit, HIF-1 $\alpha$  (hypoxia inducible factor-1 alpha)/HSPs (heat shock proteins), MAPK/PKC: Mitogen-activated protein kinase/protein kinase C.

decisions.

Several studies have shown that probiotics increase the average body weight of broiler chickens even when challenged with parasitic or bacterial pathogens [109,114,124,146]. Probiotics isolated from mare and camel milk fermented products viz. *Enterococcus faecalis* SH6, *Kazachstania unispora* Y 2.2 and *Lactocaseibacillus paracasei* subsp. *paracasei* SH1 were determined to have a significant effect ( $p < 0.05$ ) on the body and carcass weight of Ross 308 chicks. The group supplemented with probiotics ( $p < 0.05$ ) showed significant improvement in the average daily gain and feed conversion ratio. Further, probiotics group was determined to have enhanced meat protein value ( $p < 0.05$ ). However, no significant variation was accorded in organoleptic quality between the control and the experimental groups fed with probiotics [147]. In a study conducted by Abdel-Hafeez et al. [101], With or without feed restriction, chickens fed probiotic-supplemented diets showed higher body weight and improved feed efficiency than chickens fed control diets. However, meal adjustments had little effect on carcass yield. Instead, it was discovered that chickens administered probiotics had higher relative weights of the liver, gizzard, proventriculus, small intestine, and Fabricius bursa [101]. Bami et al. [99] stated that by adding *Bacillus coagulans* to chicken feed, it was possible to increase the relative weights of the legs and proventriculus, while decreasing the weights of the liver, abdominal fat, and thiobarbituric acid (TBA) value of the meat. The study also showed that adding *B. coagulans* to the diet improved the pH, yellowness, and lightness values of the leg muscles. Furthermore, administration of *Bacillus* spp. probiotics has been associated with improved feed intake, normal daily gain, and increased final body weight in broilers [135]. A previous study found that adding dietary yeast and probiotic bacteria to the feed can boost crude protein content and decrease crude fat content in breast meat. Specifically, dietary yeast and a combination of dietary yeast and probiotic bacteria reduced malondialdehyde and improved glutathione peroxidase activity in breast flesh at 35 days of age. Furthermore, incorporating these supplements has been shown to increase the levels of polyunsaturated fatty acids (PUFA), n-3 fatty acids, and the ratio of PUFA to saturated fatty acids in breast meat, while reducing the ratio of n-6 to n-3 fatty acids [148]. Even oral administration of probiotics mixture encompassing *Bifidobacterium gallinarum*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *paracasei* has been determined to enhance meat quality parameters, increase live weight gains and extend the shelf life of broiler chickens [149].

Consequently, feed composition plays a critical role in determining the growth, development, and overall health of chicken. Incorporating appropriate dietary supplements can increase the productivity of healthy broiler chickens and improve their meat quality. These studies indicate that optimizing the probiotic content of feed supplements may be an effective method for achieving these objectives. Broiler chickens can achieve optimal growth and health when probiotics are carefully selected and formulated in their feed, ultimately resulting in high-quality meat.

## 5. Probiotic feeds available in the market

The lactobacilli-based poultry probiotic ingredient market is experiencing significant growth, with a projected global market value of 80 million dollars by 2027 and a compound annual growth rate (CAGR) exceeding 6 % [150]. One example of a probiotic feed additive that has gained approval is “BioPlus 2B,” approved by the European Commission in 2003. *Bacillus licheniformis* CH 200 and *Bacillus subtilis* CH 201 were both included in this feed additive. It complies with Council Directive No. 70/524/EE C’s (EFSA 2003) requirements for animal nutritional additives, products, and substances. High levels of proteases, lipases, and amylases produced by these bacterial species in the small intestine are known to promote metabolism and improve nutrient absorption. Probiotic strains, such as *Bacillus subtilis*, produce volatile fatty acids in addition to their enzymatic activity, which can decrease the pH of the

digestive system. While preventing the formation of pathogenic *E. coli*, this acidic environment encourages the establishment of lactacidogenic microflora [151]. Chr. Hansen, a Denmark-based company, has developed a thermostable in-feed probiotic strain of *Bacillus subtilis* under the tradename GalliPro®. This engineered probiotic was designed to promote balanced gut microflora in broilers and has been approved by the EU [152]. Another company in Canada developed a probiotic-based feed under the brand name Gallina+. This feed contained dehydrated cultures of various probiotic strains, including *Bacillus subtilis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* [153]. Sukafeed-Biotechnology Co. Ltd. (China) produces a commercial biological feed probiotic called “SUKAFEED-W. Gain”. This probiotic contains strains of *Bacillus subtilis*, *Lactobacillus acidophilus*, and others [154]. It helps maintain the gastrointestinal ecological balance by inhibiting the growth of harmful bacteria and effectively breaking down starch, protein, and other complex compounds in feed into small-molecule nutrients that are easily digestible and absorbable by poultry. As a supplement, it improves feed utilization, enhances weight gain, and increases the production performance of poultry. Another commercially available probiotic is “Lactoplus,” produced by Sujay biotech Pvt. Ltd. in India. It is a live microbial feed supplement containing lactic acid bacteria that helps improve intestinal microbial balance [155]. Primalac, a USA-based company, produces a probiotic called “Primalac Poultry W/S.” This probiotic contains *Lactobacillus Casei*, *Lactobacillus acidophilus*, *Enterococcus faecium*, and *Bifidobacterium thermophilum*. The use of these strains has led to an improvement in intestinal microbial balance and an increase in poultry production performance [156].

## 6. Role of probiotic fermented products in chicken feed

Several plant-based raw materials, such as fresh vegetables, harvests, mushrooms, marine algae, and Chinese herbal treatments, can be fermented to produce fermented plant products (FPP) using probiotics such as *Lactobacillus*, yeasts, and molds. Probiotics, prebiotics, biological enzymes, and a variety of bioactive substances such as polyphenols, secondary metabolites, and other useful substances are produced during fermentation process [157,158]. FPP has been found to have several beneficial properties and plays a role in modulating the gut microbiota through its probiotic constituents. Additionally, FPP exhibits antioxidant, antibacterial, and anti-inflammatory properties, which contribute to its effects on various physiological processes. FPP has also been reported to enhance immunity and improve intestinal barrier function, which are important for maintaining overall health (Fig. 2) [159–162]. In summary, FPP derived from the fermentation of plant-based raw materials by probiotics offers a range of bioactive compounds and functional components that positively affect gut microbiota, providing antioxidant and anti-inflammatory effects, enhancing immunity, and enhancing gut barrier performance.

Fermented soybean meal supplemented with *Lactobacillus casei* has been determined to improve the average daily feed intake (ADFI), average daily gain (ADG) and villus height (VH)/crypt depth (CD) in the duodenum and rectum of broiler chickens. The fermented soybean meal also upregulated the mRNA expression levels of interleukins as well as tight junction proteins that are responsible for modulating the redox status of the ileal mucosa [163]. In another study, feeding of citrus pomace fermented by probiotics significantly enhanced ( $p < 0.05$ ) the final-body-weight and average-daily-gain of broilers as well as increased the levels of polyunsaturated fatty acids (PUFAs) and n-6 PUFAs [164]. Sun et al. [165] investigated the effects of fermented plant fraction (FPF), which is generated from solid, complete feed based on maize, soybean meal, cottonseed meal, and rapeseed meal, and is mediated by *Lactobacillus* and *Bacillus subtilis*, on various grill chicken traits. They investigated parameters such as performance in terms of development, nutrient usage, meat quality, antioxidant levels, and gut microbiota. The results of the study indicated that the inclusion of 5 % and 10 % FPF in

the feed significantly positively affected the growth performance of broiler chickens during the 1–42-day period. Additionally, the inclusion of 15 % FPF in the feed improved the birds' metabolic rates during the 19-to 21-day period. Furthermore, the inclusion of FPF in the feed led to an increase in the concentration of monounsaturated fatty acids and a notable reduction in cholesterol content in chickens. These findings suggest that FPF supplementation could affect the fatty acid composition and cholesterol levels of broiler chicken meat, with implications for meat quality and consumer health. The results of this investigation were attributed to the combined effects of probiotics and plant sources used in FPF. Specific strains of probiotics and plant materials used in the fermentation procedure likely contributed to the observed results. This study highlights the potential benefits of incorporating FPF from various plant sources and fermentation with specific probiotic strains into broiler chicken feed. However, the mechanisms underlying these effects should be investigated further to optimize the use of FPF in poultry production.

Ding et al. [166] investigated the effects of feeding broiler chickens with probiotics (*Lactobacillus*, *Saccharomyces* and *Bacillus subtilis*)-based fermented mulberry leaf powder (FMLP) on various aspects of gut health, nutrient digestibility, growth performance, and meat quality. Incorporating FMLP, which is fermented using probiotics, into the diet of grill chickens resulted in several positive effects. These include an increased villus height-to-crypt depth ratio in the gut, indicating improved gut health and nutrient absorption. The enhanced performance of gut amylase led to easier digestion of dry matter and crude protein. Furthermore, including FMLP in the diet improved ADG and reduced the feed-to-gain ratio (F/G), enhancing broiler growth performance and feed efficiency. Supplementation with 3 % FMLP in the diet also led to increased breast muscle yield, decreased abdominal fat ratio, and improved slaughter performance in broilers. Regarding meat quality, FMLP supplements enhanced the information on inosine monophosphate, total amino acids, necessary amino acids, and delightful amino acids in both thigh and breast muscles. It also improves the levels of PUFA and essential fatty acids in breast muscle [166].

Park et al. [167] demonstrated the beneficial effects of supplementing broiler feed with 1 % fermented ginger stems using *Lactobacillus paracasei*. Supplementation resulted in increased body weight and feed intake and reduced mortality when the broilers were challenged with *Salmonella gallinarum*. Additionally, the dietary fermented ginger stem led to decreased cecal *Salmonella* spp. counts and serum IgA and IgG levels in broilers, indicating potential antimicrobial and immunomodulatory effects. Sun et al. [168] investigated the effects of incorporating fermented cottonseed meal, fermented using *Bacillus subtilis* BJ-1, into the feed of yellow-feathered broilers. The inclusion of fermented cottonseed meal improved growth performance, cecal microflora, intestinal morphology, and digestive enzyme activity in broilers. Furthermore, incorporating fermented milk products such as kefir, yogurt, and probiotics into broiler feed has also been shown to positively affect performance. Ghasemi-Sadabadi et al. [169] demonstrated that including fermented milk products improved broiler feed intake, BWG, and FCR. Overall, these studies indicate that incorporating fermented products, such as fermented ginger stems, fermented cottonseed meal, and fermented milk products, in the broiler feed, can have significant effects on carcass yield, intestinal length, thigh yield, and abdominal fat in male and female chickens.

## 7. Concept of paraprobiotics and their role in chicken feeds

The term “paraprobiotics” or “ghost probiotics” was coined by Taverniti and Guglielmetti in 2011 to refer to non-viable microbes, either in intact or disintegrated form (such as crude microbial extracts), that can still have a positive impact on the consumer, whether human or animal [170]. Paraprobiotics have been found to possess various beneficial components derived from the cell envelope of bacteria, including surface proteins, peptidoglycans, cell wall polysaccharides, and

cytoplasmic components. These constituents contribute to several positive effects, such as barrier preservation and anti-tumor, immunomodulatory, and antimicrobial properties, when consumed by the host (Fig. 2) [171–174]. El-Ghany [175] explored how paraprobiotics function as natural antibiotics and highlighted their significance in poultry feed.

Paraprobiotics are generated by inactivating viable probiotic microorganisms using various techniques including heat inactivation, ionizing radiation, ultraviolet (UV) rays, high-pressure treatment, and sonication [176]. Heat inactivation is the most commonly employed method for preparing paraprobiotics [177]. Each inactivation method results in the death of microorganisms via different mechanisms, as depicted in Fig. 3. However, when producing paraprobiotics, it is important to ensure that the beneficial attributes of probiotic microorganisms are retained, even after inactivation [178]. Paraprobiotics must retain the beneficial attributes of their microorganisms to maintain positive effects on consumers. The optimal method for inactivation, functionality, and stability of the produced paraprobiotics will require extensive research and testing.

Khonyoung and Yamauchi's [179] reported that heat-killed *Lactobacillus sakei* HS-1 could promote the hypertrophication of intestinal absorptive epithelial cells on the apical surface of the villus. Therefore, the chickens were more resistant to environmental stress, resulting in improved growth performance in broiler chickens. Incharoen et al. [180] and Thi et al. [181] investigated the effect of heat executed *Lactobacillus plantarum* L-137 (HK L-137) supplementation on broiler chickens. Supplementing 10 and 50 mg of HK L-137 paraprobiotic per kilogram of feed during the first 22–28 days, significant improvements in BW, ADG, and FCR were observed, however, no significant changes were observed in feed intake, survival rate, carcass characteristics, and visceral organs.

In studies conducted by Incharoen et al. [180] and Thi et al. [181] found that a high dosage of paraprobiotics in broiler feed had certain effects on the immune response and intestinal parameters of birds. Specifically, at 22–28 days of age, the gene expression levels of the spleen index value and cytokines (including interleukin-12 subunit  $\beta$  (IL-12 $\beta$ ), interferon  $\beta$  (IFN- $\beta$ ), and interferon gamma (IFN- $\gamma$ )) increased in broilers fed with a high dosage of paraprobiotics. In terms of intestinal parameters, a study conducted by Incharoen et al. [180] showed that at 22 days of age, broilers fed paraprobiotics had increased villus height, villus area, and the ratio of villus height to crypt depth. However, no specific changes in the gut parameters were observed in the dietary groups at 42 days of age. Tukaram et al. [182] studied the effects of heat-killed *Lactobacillus acidophilus*, a dietary paraprobiotic, on 240-day-old CARIBRO Vishal commercial broiler chickens. They examined the effect of supplementation with different proportions of paraprobiotics on grill performance, immunity, intestinal health, and carcass features. The study showed that when the feed was supplemented with 0.6 % and 0.8 % paraprobiotics, significant changes were observed in various parameters. Broilers exhibited increased BWG, decreased feed intake, and improved FCR. Furthermore, the same proportion (0.6 % and 0.8 %) of paraprobiotic supplementation also led to an increased cellular defense and hemagglutination titer (HA titer). This study concluded that the addition of paraprobiotics at a rate of 0.6 % (w/v) to broiler diets could improve productivity, immunity, intestinal well-being, and breast yield [182]. The results of these studies indicate that heat-killed *Lactobacillus acidophilus* may be beneficial as a paraprobiotic for broiler production.

Zhu et al. [183] investigated the effects of dual-strain paraprobiotics, also known as compound paraprobiotics, on the gut microflora of yellow-feathered broilers. They used inactivated *Lactobacillus acidophilus* and *Bacillus subtilis* by heat treatment as the two strains for supplementation. The study revealed that supplementation with 500 mg/kg paraprobiotics in the broiler basal diet improved feed efficiency throughout the 1–63-day period. Additionally, paraprobiotics significantly influenced the composition of gut microflora. At 21 days of age, increasing the relative abundances of *Barnesiella* sp. and *Lactobacillus*

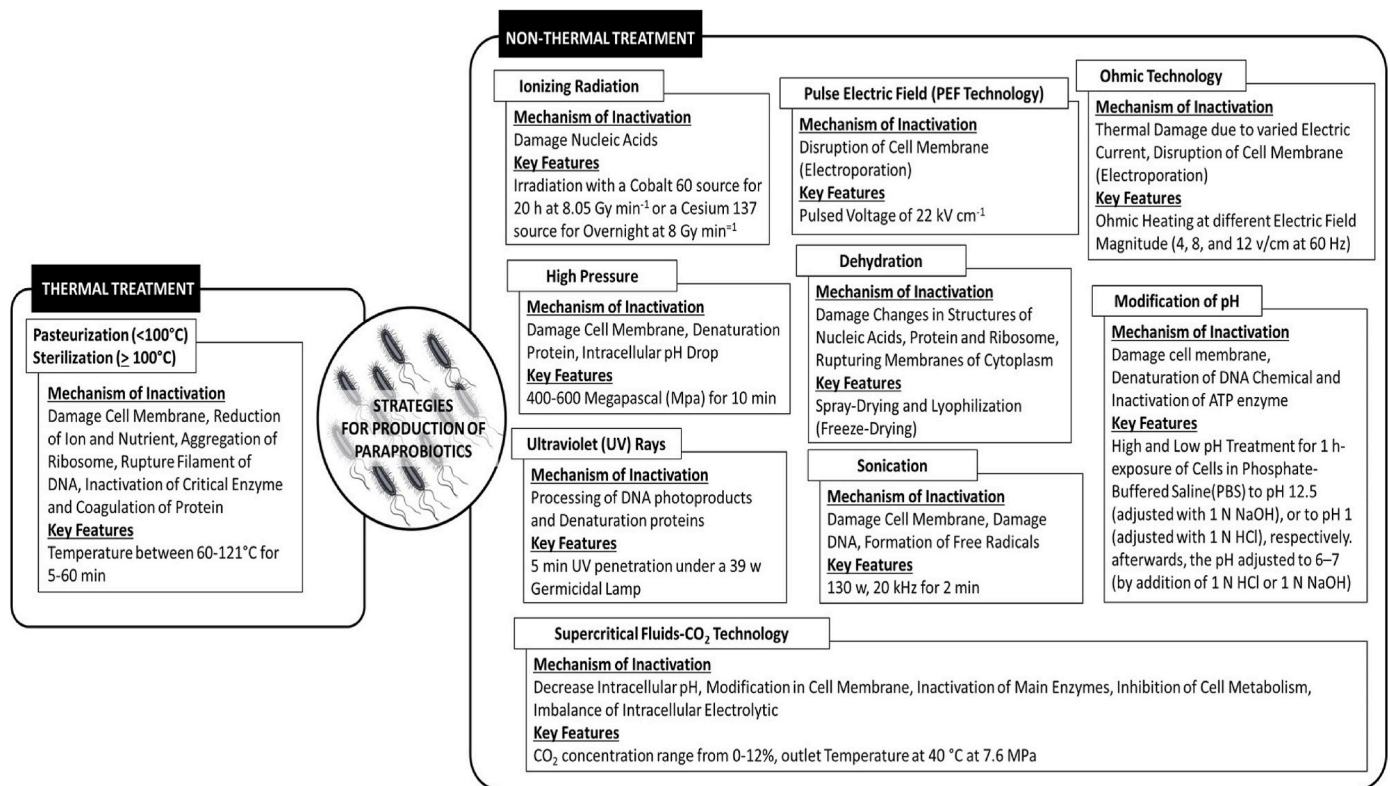


Fig. 3. Technical strategies employed for the production of paraprobiotics.

*aviaries* indicated a shift in the composition of the microbial community, specifically in favor of these two bacterial species. At 42–63 days, there was a reduction in the population of microbes belonging to the *Lachnospiraceae* family, as well as the *Lachnoclostridium* and *Peptococcus* genera [183]. In a recent study conducted by Danladi et al. [184], they investigated the effects of dietary treatment with paraprobiotics derived from *Lactiplantibacillus plantarum* on one-day-old (COBB 500) chicks. They specifically examined the impact on colonic mucosa secretory Immunoglobulin A (sIgA) and plasma immunoglobulin IgM levels during the finisher phase. The study revealed significant ( $p < 0.05$ ) changes in colonic mucosal sIgA and plasma immunoglobulin IgM levels following dietary treatment with paraprobiotics. Although further studies are necessary to gain a better understanding of how paraprobiotics affect broiler growth and health, these findings are significant for establishing paraprobiotics as a potential alternative feed additive or supplement.

## 8. Limitations of the study

The majority of studies discussed successfully comprehended the health-promoting impact of single or mixed genera of probiotics on the overall health of chickens. However, none of the studies explored the interaction mechanism between the feed additives and probiotics. Moreover, the concept of paraprobiotics again has been determined to be beneficial for broilers health. Still, the factors that needs to be taken in consideration while introducing such microbes in functional feed of broiler have not been address in our study. Additionally, review by Krysiak et al. [24] highlighted that prolong use of probiotics could lead to their elevated concentrations in the cloaca which would potentially result in deteriorated semen quality.

## 9. Conclusion

In light of the prohibition of certain antibiotics in animal production,

research has been conducted to find safer alternatives that can improve the productivity and condition of broilers without endangering human health. These alternative options should possess a range of beneficial properties in order to provide comprehensive benefits. Given the specific requirements for alternative feed additives in broiler production, probiotics, paraprobiotics, and probiotic-fermented products have emerged as prominent options. Extensive research has been conducted that focused on understanding the role of probiotics as functional feed in broilers which serves the function of natural antibiotics as well as growth promoters. Additionally, probiotics fermented plant-based feed act as unique combination that improves the overall wellbeing of broilers. Finally, paraprobiotics have emerged as new functional feed that provides same benefits as compared to the live probiotics. But one area that requires investigation is the interaction effect of combining different additives and sub additives. Understanding how these additives interact with each other can help to identify synergistic effects that can further enhance their efficacy. Further studies of their interaction with dietary nutrients, efficiency of utilization, and appropriate inclusion levels need to be explored. These investigations should be considered mandatory to advance our understanding of alternative feed additives in broiler production. Last but not the least, there is dire need for in-depth investigation to establish the role of paraprobiotics in broiler feeds.

## CRedit authorship contribution statement

**Harsh Kumar:** Writing – original draft, Visualization, Validation, Conceptualization. **Indu Bhardwaj:** Writing – original draft, Investigation. **Eugenie Nepovimova:** Writing – original draft, Validation, Supervision, Project administration, Methodology. **Daljeet Singh Dhanjal:** Writing – original draft, Visualization, Formal analysis. **Sumayya Sana Shaikh:** Writing – original draft, Visualization, Validation. **Renáta Knop:** Writing – original draft, Visualization, Validation. **David Atuahene:** Writing – original draft, Visualization, Validation.

**Ayaz Mukarram Shaikh:** Writing – original draft, Visualization, Software, Methodology, Data curation. **Kovács Béla:** Writing – original draft, Visualization, Supervision, Resources, Project administration, Formal analysis.

### Ethics approval statement

None.

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None.

### Funding

Project No. TKP2021-NKTA-32 was implemented with support from the National Research, Development, and Innovation Fund of Hungary, financed under the TKP2021-NKTA funding scheme and Supported by the University of Debrecen Program for Scientific Publication.

### Declaration of competing interest

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

### Acknowledgment

This work was supported by the Czech Republic (Excellence Project PrF UHK 2208/2024–2025), MH CZ- DRO (UHHK, 00179906).

### Data availability

All data were collected from the published research papers.

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