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«Review»

Regulatory Effects of the Probiotic *Clostridium butyricum* on Gut Microbes, Intestinal Health, and Growth Performance of Chickens

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Clostridium butyricum is an important probiotic for chickens and exerts various biological activities, including altering the composition of the intestinal microbiota, competing with other microorganisms for nutrients, improving the integrity of the intestinal mucosal system, changing the intestinal barrier, and improving overall host health. Intestinal microbes also play vital roles in maintaining the intestinal barrier, regulating intestinal health, and promoting chicken growth. During chicken production, chickens are vulnerable to various stressors that have detrimental effects on the intestinal barrier with significant economic consequences. *C. butyricum* is a known probiotic that promotes intestinal health and produces the short-chain fatty acid butyric acid, which is beneficial for the growth performance of chickens. This review elucidates the development and utilization of *C. butyricum* to improve intestinal barrier function and growth performance in chickens through its probiotic properties and interactions with intestinal microbes.

Key words: *Clostridium butyricum*, chicken, gut microbes, intestinal barrier, probiotic

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Introduction

Alongside the prohibitions on the use of antibiotic growth promoters in the chicken industry, there remain multiple stressors that cause gastrointestinal health problems in chickens (Liang *et al.*, 2021). Therefore, there is an urgent need to find efficient alternatives to antibiotics to promote animal health and reduce economic losses in the chicken industry. Probiotics are living microorganisms that play beneficial roles in animals. In particular, probiotics interact with the gut microbiota of chickens to exert important functions, such as growth and development (Feng *et al.*, 2021), nutrient metabolism (Shehata *et al.*, 2022), substance absorption (Ramírez *et al.*, 2022), and immune regulation (Mindus *et al.*, 2021). In addition to their primary function in establishing gut homeostasis, probiotics exert positive effects by improving immunity (Jiang *et al.*, 2021), reducing excess lipid accumulation (Chen *et al.*, 2021), enhancing egg quality (Zhan *et al.*, 2019), and increasing the production performance (Zhang *et al.*, 2021a) of chickens. Along with the direct effects of probiotics, short-chain fatty acids (SCFAs), which are important metabolites derived from intestinal microbial fermentation, act on the intestinal tract of poultry to improve gut health, strengthen immunity, and enhance the growth performance of birds (Zhang *et al.*, 2012; Yosi *et al.*, 2022). Therefore, probiotics are potential targets for modifying the intestinal health and production performance of chickens.

Clostridium butyricum, a natural gram-positive probiotic (Bassiony *et al.*, 2021; Fu *et al.*, 2021), possesses a variety of biological activities, including reshaping of the gut microbiota, inhibition of pathogenic bacteria, anti-inflammation, and immune regulation, which contribute to the improved performance of chickens (Hossain *et al.*, 2015; Han *et al.*, 2018; Wang *et al.*, 2022a). In addition, butyrate, the main metabolite of *C. butyricum*, has beneficial effects on the proliferation of intestinal cells (Friedel and Levine, 1992) and production of gastrointestinal hormones (Mineo *et al.*, 1994). In this review, the regulatory ef-

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fects of *C. butyricum* on the gut microbiota, intestinal health, immunity, and growth performance of chickens are elucidated.

Beneficial Properties and Applications of Probiotics in Chickens

Effectiveness of probiotics and their influencing factors

Probiotics are live microorganisms that promote the growth of beneficial bacteria, modify the structure of intestinal microorganisms, and substantially contribute to animal health (Vilà *et al.*, 2010; Morelli and Capurso, 2012). In recent years, several studies have confirmed that developing green alternatives to antibiotics can solve various intestinal problems and maintain the balance of intestinal microorganisms, ultimately improving the health and production performance of poultry (Xiao *et al.*, 2021; Agustono *et al.*, 2022; Kulkarni *et al.*, 2022; Luise *et al.*, 2022). The effectiveness of probiotics can be affected by the species of microbes, amount added, and form of additives used (Khan *et al.*, 2019; Zhang *et al.*, 2021b). Currently, the commonly used probiotics in poultry farming include *Bifidobacteria* (El-Sharkawy *et al.*, 2020), *Lactobacillus* (Gilliland, 1990), *Enterococcus faecalis* (Shehata *et al.*, 2020), *Bacillus licheniformis* (Zhao *et al.*, 2020b), *Bacillus subtilis* (Memon *et al.*, 2022), *Streptococcus thermophilus* (Sihite and Pramono, 2022), and *Aspergillus oryzae* (Chuang *et al*., 2019), among others. Different types of probiotics have different regulatory effects on animal health and performance. Reuben *et al.* (2022) evaluated mono-strain probiotics (*Pediococcus acidilactici* I5, *Pediococcus pentosaceus* I13, *Enterococcus faecium* C14, *Lactobacillus plantarum* C16, with commercial probiotics as positive control) and the effects of these probiotic mixtures on the performance of broilers. They found that *P. pentosaceus* I13, *L. plantarum* C16, and multi-strain probiotics remarkably improved body weight gain and the feed conversion ratio. This growth-improving action may be attributed to intestinal enterobacterial counts. The beneficial effects of probiotics are also influenced by their form. For example, probiotics delivered to the distal part of the gut show better protective actions in chickens. Gyawali *et al.* (2022) found that 500 ppm of encapsulated *Lactobacillus paracasei* increased the villus height/crypt depth ratio, *ZO-1* expression, and levels of anti-inflammatory cytokines and further increased the abundance of *Bacteroides*. However, additional research is needed to discover novel strains and appropriate amounts that would elicit probiotic actions to promote the healthy development of chickens.

Probiotics interact with the gut microbiota to exert beneficial effects

Probiotics exert beneficial effects by interacting with the gut microbiota, which is important for the growth and development of chickens (Khan *et al*., 2019; Madlala *et al*., 2021; Memon *et al*., 2022). For example, dietary supplementation with *Lactobacillus salivarius* CML352 altered the gut microbiota by reducing the Firmicutes/Bacteroidetes ratio and further increased *Muc2* expression and influenced late-phase laying hens' intestinal health positively (Xu *et al.*, 2022). Additionally, probiotics compete with pathogenic bacteria for nutrients from the host and mucosal adhesion sites, thus inhibiting the propagation of harmful bacteria, such as *Escherichia coli* and *Salmonella*. Lee and Bak (2011) reported that probiotics can enhance the integrity of the intestinal physical barrier, maintain immune tolerance, and reduce pathogen translocation across the intestinal mucosa. Memon *et al.* (2022) found that dietary supplementation with *B. subtilis* enriched the abundance of some commensal genera in chicks injected with *Eimeria tenella*, such as *Clostridium sensu stricto 1*, *Corynebacterium*, *Enterococcus*, *Romboutsia*, *Subdoligranulum*, *Bacillus*, *Turicibacter,* and *Weissella*. An increased abundance of these gut microbes promoted butyrate production, stimulated anti-inflammatory responses, and protected against pathogens, thereby alleviating *E. tenella*-induced intestinal disruption. However, Yang *et al.* (2022b) observed that pretreatment with the probiotic *Akkermansia muciniphila* exacerbated the loss of species richness in broilers challenged with *Eimeria* and *Clostridium perfringens*. Therefore, the application of some probiotic strains in chickens should be performed with caution, as dietary probiotics change and reshape the composition of microorganisms; thus, they are integral to regulating intestinal microorganisms.

Prebiotic effects of probiotics by producing intestinal metabolites

In addition to the direct effects of probiotics on chicken physiology, they produce various metabolites, including SCFAs (Ito *et al*., 2022; Ricke, 2003), organic acids (Herzallah, 2013), bacitracin (Smialek *et al.*, 2018), and enzymes (Kalathinathan and Kodiveri Muthukaliannan, 2021; Shehata *et al.*, 2022), which can regulate host metabolism. Enzymes are among the main intestinal metabolites that promote growth and improve the application of probiotics (Zhang *et al.*, 2021b). Probiotics colonize the gut lumen and stimulate the release of digestive enzymes, thereby improving nutrient absorption and promoting the growth performance of birds (Shehata *et al.*, 2022). Kalathinathan and Kodiveri Muthukaliannan (2021) reported that the enzyme β-galactosidase extracted from the isolate *Paracoccus marcusii* KGP hydrolyzed 47% of whey lactose efficiently at 50 °C, which was subsequently used to produce animal feed. SCFAs, including acetate, propionate, and butyrate, are another class of important metabolites that act as bacteriostatic agents against foodborne pathogens (Ricke, 2003). The primary metabolic products of *Bifidobacteria* are acetate and lactate, which inhibit the invasion of pathogenic microbes (Plaza-Diaz *et al.*, 2019). After broilers were continuously supplanted with *Weizmannia coagulans* SANK70258, the fecal metabolites were found to be altered, along with increased propionate and butyrate contents, which may be associated with growth-promoting functions (Ito *et al.*, 2022). Dietary supplementation with *Lactobacillus* and *Rhodobacter capsulatus* could increase polyunsaturated fatty acid concentrations and reduce cholesterol levels in chickens (Selma *et al.*, 2007; Kalavathy *et al.*, 2008; Ramasamy *et al.*, 2009; Herzallah, 2013). In addition, *Bacillus licheniformis* secretes the natural polypeptide antibiotic bacitracin (Anthony *et al.*, 2009; Smialek *et al.*, 2018).

Probiotics promote the growth performance of chickens through the microbiota–gut–tissue axis

The beneficial effects of probiotics and their associated metabolites on intestinal health promote the growth performance and body health of chickens via the microbiota–gut–tissue axis. Egg quality is an important index related to the economic value of laying hens and can be improved by probiotic administration (Xiang *et al*., 2019; Wang *et al*., 2020a; Macit *et al*., 2021; Marwi *et al*., 2021). Probiotic strains such as *Lactobacillus fermentum* (Palaniyandi *et al.*, 2020), *Limosilactobacillus reuteri* (Liu *et al.*, 2022b), *Lacticaseibacillus rhamnosus* (Zafar *et al.*, 2022), and *Lactobacillus acidophilus* (Abdulrahim *et al.*, 1996) are known for their ability to remove cholesterol. For example, *L. salivarius* CML352 could reduce abdominal fat deposition and improve egg quality in late-phase laying hens (Xu *et al*., 2022). Khogali *et al.* (2022) found that a 4-week treatment with *C. butyricum* and *B. subtilis* decreased the pimpled or sandpaper-shelled egg rate from 42.51% to 28.02% in 450-d-old Hy-Line hens. In broilers, bone health is essential for welfare and production performance (Jansen *et al.*, 2020; Jiang *et al.*, 2021). *Bacillus amyloliquefaciens* CGMCC18230 supplementation improved tibial bone mineralization and growth performance by increasing the relative abundances of *Ruminococcaceae* (butyrate-producing), *Akkermansia* (polyamine-producing), and *Alistipes* (polyamine-producing) (Li *et al*., 2022). Previous studies have also shown that probiotics exert beneficial effects on the muscle growth of broiler chickens (Shah *et al.*, 2019; Stasiak *et al.*, 2021). Dietary supplementation with *Enterococcus faecium* AL41 increased the number of myonuclei per fiber, improved capillarization, and further improved body weight owing to the high intramuscular expression of *IGF-1* and lowered *MYF5* expression in broilers (Albrecht *et al.*, 2022). In addition, microbiota dysbiosis may induce neuroinflammation via the microbiota–gut–brain axis, which causes injurious behaviors in chickens (Jiang *et al.*, 2022; Shamshirgaran *et al.*, 2022). Jiang *et al.* (2022) showed that the probiotic *B. subtilis* reduced stress-induced injurious behavior by mediating the gut–microbiota–brain axis.

In summary, probiotics can maintain intestinal health by competing with other microorganisms for nutrients, improving the integrity of the intestinal mucosal system, binding to adhesion sites on the intestinal mucosa to reduce pathogen colonization and infection, changing the intestinal barrier, regulating the composition of the intestinal microbiota, promoting the growth of intestinal epithelial cells, producing metabolites, and improving the overall health of the host. These probiotics are summarized in Table 1, and their mechanisms of action are summarized in Figure 1.

In addition, some probiotics act synergistically with other probiotics or natural additives on the gut microbiota to promote animal health (Rodjan *et al.*, 2018; Hassan *et al.*, 2022; Khogali *et al.*, 2022; Madne *et al.*, 2022; Reuben *et al.*, 2022). Supplementation of a *C. butyricum* and *B. subtilis* mixture improved intestinal development by increasing the villus length and ratio of villus length to crypt depth (Khogali *et al.*, 2022). Another study found that the combined supplementation of organic acids and probiotics increased the ability of broilers to digest crude fiber and villus height while inhibiting the growth of *E. coli* (Rodjan *et al.*, 2018). Therefore, dietary intervention is an important method for modifying gut microbes, regulating intestinal barrier function, and achieving significant improvements in chicken production performance (Mikulski *et al.*, 2020; Macit *et al.*, 2021; Popescu *et al.*, 2021; Moon *et al.*, 2022; Obianwuna *et al.*, 2022; Qiu *et al.*, 2022). Accordingly, research and development of novel prebiotic and probiotic products are important for the chicken industry. Although various novel probiotics for chickens still need to be identified, in-depth research on the relevant mechanisms of these probiotic products is lacking.

C. butyricum Interacts with Gut Microbes to Regulate Intestinal Health

The probiotic C. butyricum is a potentially effective antibiotic alternative

The ban on the use of antibiotic growth promoters as feed additives has exacerbated intestinal health problems (especially gut microbiota dysbiosis) and caused economic losses in the chicken industry (McEwen *et al.*, 2018; Redondo *et al.*, 2022). The composition of the intestinal microbiota in chickens is influenced by various factors, including diet, gastrointestinal region, environment, and genetics, among which dietary intervention is the most direct and effective method for regulating intestinal microbiota (Ding *et al*., 2017; Kers *et al*., 2018). Although the composition of intestinal microbes at different developmental stages is tightly regulated, the developmental time and succession of intestinal microbes are affected by various factors (Pandit *et al*., 2018; Ngunjiri *et al*., 2019). Studies have confirmed the influence of production management, diet, disease, genetics, and other factors on the composition of gut microbes (Yadav and Jha, 2019; Popescu *et al.*, 2021; Upadhaya *et al.*, 2021; Jia *et al.*, 2022; Liu *et al.*, 2022a). Importantly, there is an urgent demand for green antibiotic substitutes in the chicken industry following widespread concerns regarding antimicrobial resistance and consequent antibiotic bans. Therefore, *C. butyricum*, as a well-known gram-positive and obligate anaerobic bacillus that has been confirmed to exert probiotic properties, could serve as a potential alternative for modulating gut health. *C. butyricum* provides beneficial properties to animals through its actions on the intestine and its interaction with the gut microbiota; therefore, it is recognized as a probiotic (Duan *et al.*, 2018; Huang *et al.*, 2019; Wang *et al.*, 2020b). Compared to other probiotics such as *Lactobacillus* and *Bifidobacterium*, *C. butyricum* has been used for the treatment of a wide range of intestinal diseases in veterinary practice, providing a better option for the development of chicken probiotic additives because of its ability to produce endospores to survive harsh environments (extreme pH and temperature) (Douglas *et al.*, 1973; Kong *et al.*, 2011; Liao *et al.*, 2015). Moreover, studies have indicated that *C. butyricum* probiotics may be a better alternative to conventional antibiotics in chickens. For example, Zhang *et al.* (2016) evaluated the probiotic effects of *C. butyri-*

Table 1. **Summary of effects and possible mechanisms of probiotics on chickens**

Probiotics	Dosage	Animals	Effects and possible mechanisms	Reference
Pediococcus pentosaceus I13; *Lactobacillus plantarum* C16	1×10^8 CFU/mL/chick (oral gavage)	1-day-old Cobb 500 broilers	Remarkable improvement in body weight gain and feed conversion ratio, which may be due to the intestinal *Enterobacteria* counts.	Reuben *et al.* (2022)
Lactobacillus paracasei	500 ppm encapsulated probiotic	1-day-old Arbor acres broilers	Increased the villus height/crypt depth, *ZO-1* expression, and levels of anti-inflammatory cytokines, and further increased the abundance of *Bacteroides*.	Gyawali *et al.* (2022)
Lactobacillus salivarius CML352	1×10^8 CFU/kg feed	65-week-old Hy-Line Brown laying hens	Increased *Muc2* expression and influenced late-phase laying hens' intestinal health, reduced abdominal fat deposition and improved the egg quality of late-phase laying hens.	Xu *et al.* (2022)
Bacillus subtilis	1×10^8 CFU/kg feed	Newly hatched chicks	Enriched the abundance of some commensal genera in chicks injected with *Eimeria tenella*.	Memon *et al.* (2022)
Akkemansia muciniphila	1×10^9 CFU/bird (oral gavage, 1 mL/chick)	1-day-old Cobb broiler chicks	Loss of species richness in broilers challenged with *Eimeria* and *Clostridium perfringens*.	Yang *et al.* (2022b)
Weizmannia coagulans SANK70258	1×10^6 CFU/mL in drinking water (first 10 days) and thereafter at least 10^6 CFU/g in feed	Broiler chicks (10 days after birth)	Growth-promoting effect and increased propionate and butyrate contents in broilers.	Ito *et al.* (2022)
Lactobacillus	1×10^6 CFU/mL (oral gavage)	1-day-old broiler chickens, 23-week-old layer hens	Increased polyunsaturated fatty acid concentration and reduced cholesterol levels in chickens.	Herzallah (2013)
Lactobacillus acidophilus	1×10^6 CFU/kg feed	12-week-old Lohmann-white shell laying hens	Cholesterol removal ability	Abdulrahim *et al.* (1996)
Bacillus amyloliquefaciens CGMCC18230	2.5×10^{10} CFU/kg feed	Newly hatched, male, Arbor Acres broilers	Improved tibia bone mineralization and growth performance by increasing the relative abundance of *Ruminococcaceae* (butyrate-producing), *Akkermansia* (polyamine-producing), and *Alistipes* (polyamine-producing).	Li *et al.* (2022)
Enterococcus faecium AL41	1×10^9 CFU/0.2 mL/chick	1-day-old Cobb 500 male chicks	Elevated the myonuclei number per fiber, improved capillarization, and further improved body weight due to the higher intramuscular expression of *IGF-1* and lowered *MYF5* expression in broilers.	Albrecht *et al.* (2022)
Bacillus subtilis	250 ppm (1×10^6 CFU/g feed)	24-week-old hens	Reduced stress-induced injurious behavior by mediating the gut–microbiota–brain axis.	Jiang *et al.* (2022)
Clostridium butyricum and *Bacillus subtilis* mixture	500, 1000, and 1500 mg/kg feed *Clostridium butyricum* (1×10^8 CFU/g) and *Bacillus subtilis* (1×10^9 CFU/g).	450-d-old Hy-Line laying hens	Decreased the pimpled or sandpaper-shelled eggs rate from 42.51% to 28.02% in 450-d-old Hy-Line hens.	Khogali *et al.* (2022)

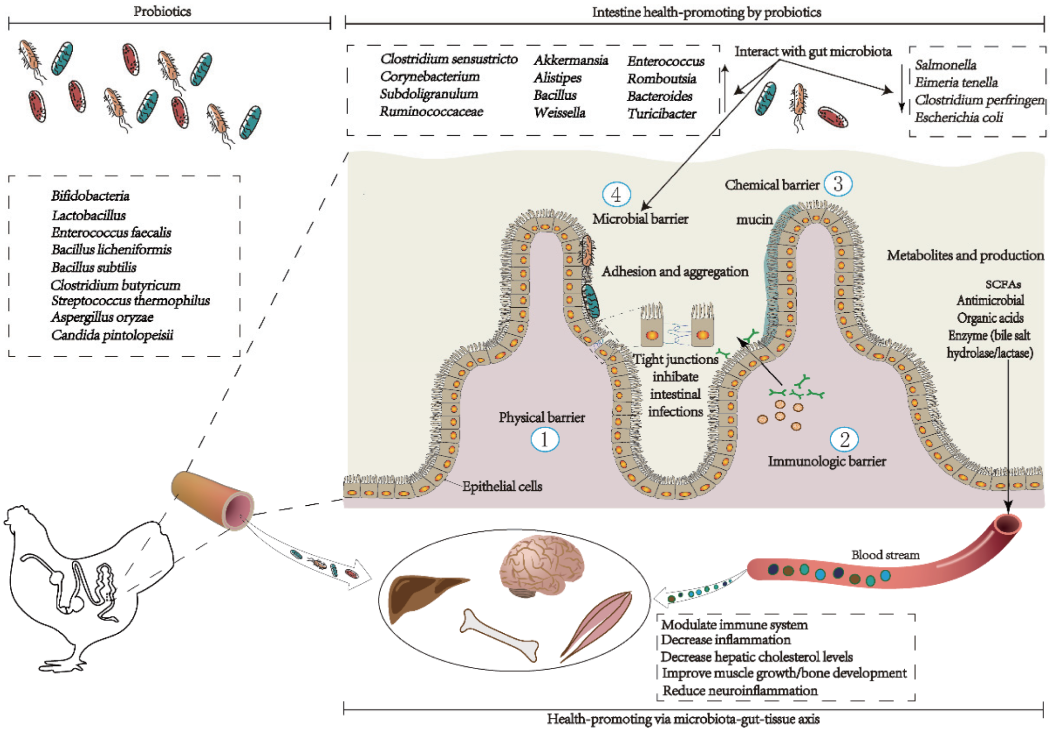

Fig. 1. **Effects of probiotics on the gut microbiota, intestinal barrier function, and host health in chickens.** Probiotics can protect and maintain intestinal health by competing with other microorganisms for nutrients, improving the integrity of the intestinal mucosal system, binding to adhesion sites on the intestinal mucosa to reduce pathogen colonization and infection changing the intestinal barrier, regulating the composition of the intestinal microbiota, promoting the growth of intestinal epithelial cells, producing metabolites, and improving the overall health of the host. In summary, probiotics enhance intestinal health, which works collaboratively to promote chicken health.

cum in broiler chickens challenged with *E. coli* K88 and found that *C. butyricum* could promote the immune response and improve intestinal barrier function; moreover, there was no remarkable difference in the effects of *C. butyricum* and colistin sulfate treatment. Yang *et al.* (2012), Zhang *et al.* (2014), Li *et al.* (2021), and Wang *et al.* (2022a) compared *C. butyricum* to various antibiotics (colistin, colistin sulfate, virginiamycin, and oxytetracycline) in broilers and found no significant differences in growth-promoting effects between the antibiotic group and *C. butyricum*-supplemented group. Another study indicated that *C. butyricum* showed more beneficial effects than aureomycin in increasing the breast muscle yield in broilers (Liao *et al.*, 2015). However, Zhang *et al.* (2011) and Han *et al.* (2018) suggested that *C. butyricum* does not affect broiler growth performance. These inconsistent findings may be due to different supplement levels, probiotic strains, or growth phases of the chickens among studies. Therefore, future studies should focus on the effective supplementation dose and timing with probiotic strains.

C. butyricum exerts beneficial effects on the composition of intestinal microbes and intestinal health

C. butyricum can affect the intestinal microbiota of chickens by directly improving the structure of the intestinal flora, increasing the abundance of beneficial bacteria, maintaining homeostasis of the intestinal environment, relieving the inflammatory response in the intestinal tract, and inhibiting the development of diseases caused by intestinal disorders (Meimandipour *et al.*, 2010; Yang *et al.*, 2012; Zhao *et al.*, 2013; Zhang and Kim, 2014). For example, 60-week-old Hy-Line Brown laying hens fed 2.7 g/kg *C. butyricum* [1.0×10^9 colony-forming units (CFU)/g] showed high *Bacteroidetes*, *Clostridia* (*Clostridiales*), and *Prevotellaceae* abundances but low abundances of *Firmicutes* and other harmful bacteria such as *Klebsiella*, thus reshaping the gut microbiota (Wang *et al.*, 2020b). Invasion by intestinal pathogens affects the composition of intestinal microorganisms and disrupts the microbial state of the host, causing an imbalance in the intestinal flora. This leads to the overgrowth of pathogenic bacteria, which can cause systemic infections (Maier *et al.*, 2013; Gloanec *et al.*, 2022; Ruvalcaba-Gómez *et al.*, 2022; Szott *et al.*, 2022). *C. butyricum* supplementation alleviated the high stocking density stress-induced intestinal dysfunction by increasing the observed species and Shannon microbial diversity indices, increasing the proportion of *Bacteroides*, and enhancing intestinal epithelial barrier function by increasing intestinal claudin-1 and *ZO-1* expression, with overall benefits for the growth performance of male Arbor Acres broilers (Li *et al.*, 2021). Therefore, improvements in the gut microbiome structure synergistically improve gut barrier function. Xu *et al.* (2021b) reported that treatment with *C. butyricum* improved intestinal health by increasing the intestinal villus height/crypt depth ratio and inducing the expression of *Muc2*, *ZO-1*, *IL-6*, and *TGF-β1*, which exerted a protective effect against intestinal damage in broilers with necrotic enteritis caused by *Clostridium perfringens*. Additionally, the gut microbiota affects nutrient digestion and absorption (Hu and Guo, 2007; Rodjan *et al.*, 2018). Supplementation with *C. butyricum* enhances nutrient absorption and retention and increases the apparent digestibility of key essential amino acids in laying hen feces (Obianwuna *et al.*, 2022), the improvement of intestinal structure and egg quality of laying hens was similar to the findings of Xu *et al.* (2022) study on *Lactobacillus salivarius* CML352. However, the properties, benefits, and effects of probiotics differ and are specific to each strain. The single-strain probiotic *C. butyricum* reportedly failed to influence the cecal microflora composition of Ross 308 broilers, whereas *Bacteroides*, *Oscillospira,* and *Faecalibacterium* were the dominant genera in the *C. butyricum*-treated group (Such *et al.*, 2019).

C. butyricum alters the metabolites of gut microbes to promote chicken health

In addition to the direct effects of *C. butyricum* on the composition of intestinal microbes and host health, this probiotic can also alter the metabolites of gut microbes. One of the most important metabolites is amylase, which plays an essential role in hydrolyzing starch and carbohydrates into oligosaccharides (Han *et al.*, 2018). The production of oligosaccharides is beneficial for growth performance (Jung *et al.*, 2008), tissue lipid accumulation (Dev *et al.*, 2021), immune function (Yuan *et al.*, 2018), and egg quality (Park and Park, 2012) in chickens. For instance, Wang *et al.* (2022b) found that chitosan oligosaccharide supplementation reduced abdominal fat deposition and drip loss in the breast muscle. An *in vitro* fermentation study compared the fermentability of six oligosaccharides using *C. butyricum* TK2 and *C. butyricum* CB8 strains. The results showed that isomalto-oligosaccharides had the strongest fermentability in both strains; however, SCFA production differed between the two strains (Wang *et al.*, 2014). Furthermore, the dietary probiotic *C. butyricum* could be found within the mucus layer, which was suggested to contribute to its action within the gut (Xu *et al.*, 2021a; Mun *et al.*, 2022). SCFAs, especially butyric acid, are produced by *C. butyricum* within the intestinal mucosa; therefore, these substances are easily accessible to hosts (Molnár *et al.*, 2020; Xu *et al.*, 2021a). Butyric acid plays an important role in nourishing intestinal goblet cells and reducing the number of intestinal pathogens (Zhang *et al.*, 2016; Guo *et al.*, 2021). However, free butyric acid is naturally volatile and has an unpleasant odor that can affect palatability and feed intake (Leeson *et al.*, 2005; Wu *et al.*, 2018). The chemical salt of butyric acid, sodium butyrate, is commonly used as a feed additive (Melaku *et al.*, 2021), and its effectiveness is limited by its rapid absorption in the gastrointestinal tract. Thus, increasing studies have focused on the protection of butyrate to facilitate the slow and gradual release of butyric acid throughout the intestine (Ventura *et al.*, 2021). *C. butyricum* has more stable beneficial effects than butyrate because of its strong adhesion to the intestine and the continuous release of butyric acid and other metabolites (Molnár *et al.*, 2020). Wang *et al.* (2021) compared the effects of *C. butyricum*, sodium butyrate, and butyric acid glycerides on yellow-feathered breeder hens and found that *C. butyricum* treatment increased the hatchability of fertilized eggs and the growth performance of offspring, whereas sodium butyrate treatment upregulated nutrient transporters in the jejunal mucosa, partially realizing the effect of *C. butyricum.* In addition, *C. butyricum* regulates other metabolic pathways in the gut, as demonstrated by metabolomic analysis. For instance, *C. butyricum* can produce postbiotics, including bacteriocins and lipoteichoic acid, which regulate antioxidation and antibacterial functions (Pan, 2006; Gao *et al.*, 2011; Junghare *et al.*, 2012).

Collectively, accumulating evidence shows that *C. butyricum* modulates intestinal microbes and produces metabolites that are beneficial for gut health in chickens. *C. butyricum* increases the butyric acid content and regulates other intestinal metabolites to achieve intestinal homeostasis, promoting both intestinal and overall host health in chickens. Therefore, the regulatory effects of *C. butyricum* on chickens appear to be related to its influence on intestinal metabolites. Moreover, the composition of intestinal microbes is complex and accounts for the distinct effects of probiotics on intestinal microbes and their associated metabolites. Hence, *C. butyricum* can increase the abundance of beneficial bacteria in the intestine, maintain homeostasis of the intestinal environment, protect the intestinal barrier, and inhibit the development of diseases caused by disorders of intestinal microbes. These mechanisms are summarized in Figure 2.

Growth-Promoting Function of *C. butyricum* in Chickens via Different Biological Activities

C. butyricum has a beneficial effect on growth performance

The use of *C. butyricum* as a dietary modifier in commercial chicken production has great potential for investigating and understanding the intestinal microbial structure and barrier functions in chickens (Svejstil *et al.*, 2019; Xu *et al.*, 2021a). Day-old Arbor Acres broilers fed *C. butyricum* exhibited improved intestinal mucosal barrier function, modified gut microbiota, and intestinal homeostasis, with beneficial effects on the growth performance of broiler chickens (Xu *et al.*, 2021a). Svejstil *et al.* (2019) confirmed that *C. butyricum* CBM 588 exerted positive effects on the body weight gain of broilers by influencing the cecal microbiota composition and increasing the amount of butyrate in the ceca. In addition to its beneficial effects on the intestinal tract, a growing number of studies have found that *C. butyricum* possesses other biological functions, such as anti-inflammation (Zhang *et al.*, 2016; Takahashi *et al.*, 2018), immunomodulatory (Yang *et al.*, 2012; Zhang *et al.*, 2014; Yang *et al.*, 2020), and antioxidation (Sun *et al.*, 2016; Yang *et al.*, 2022a) effects. These actions have been intensively studied in mammals and are gradually gaining attention in chicken nutrition research. Importantly,

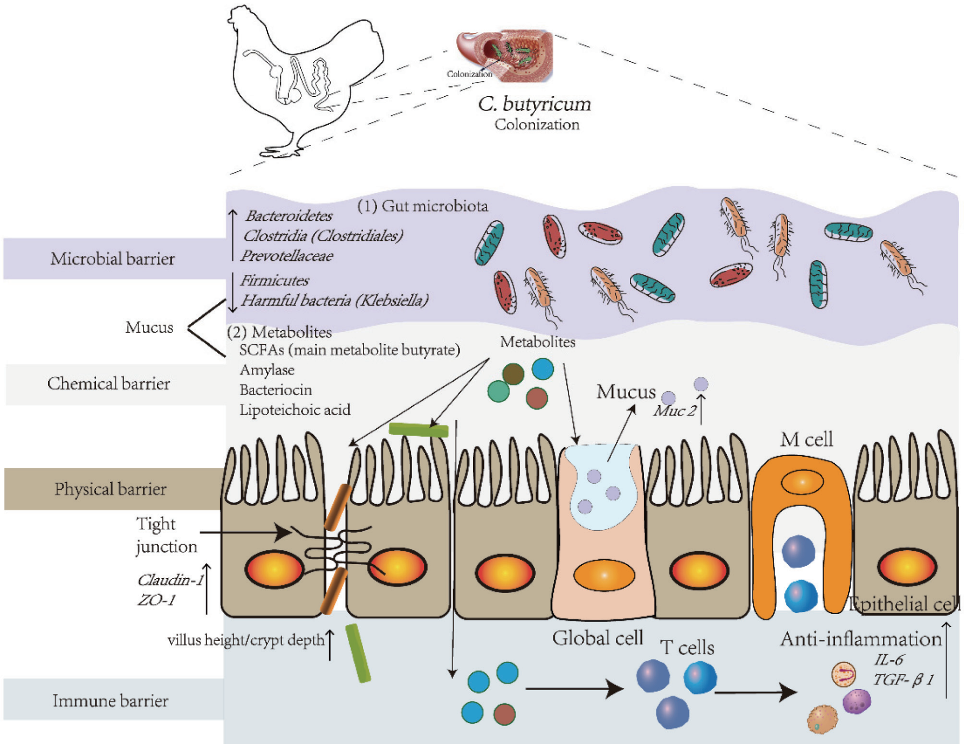

Fig. 2. **Effects and mechanisms of the probiotic *C. butyricum* on the intestinal health of chickens.** *C. butyricum* increases the abundance of beneficial bacteria in the intestine, maintains the homeostasis of the intestinal environment, protects the intestinal barrier, and inhibits the development of diseases caused by disorders of intestinal microbes.

the safety of these probiotic strains is critical for their use as antibiotic alternatives. Previous studies have confirmed the safety of *C. butyricum* and its use as a feed additive. In chickens for fatting, the safety and efficacy of the commercial probiotic Miya-Gold® S prepared from *C. butyricum* strain FERM BP-2789 were explored by the EFSA Panel on Additives and Products or Substances used in Animal Feed *et al.* (2021); they concluded that it is safe for fattening chickens at the recommended dose of 2.5 × 10^8 CFU/kg and subsequently reduced the minimum level to 1.25 × 10^8 CFU/kg feed (EFSA Panel on Additives and Products or Substances used in Animal Feed *et al.*, 2023). In addition, the EFSA Panel on Additives and Products or Substances used in Animal Feed *et al.* (2022) concluded that a mixture of probiotics, including *B. subtilis* FERM BP-07462, *Enterococcus lactis* FERM BP-10867, and *C. butyricum* FERM BP-10866, was safe as an additive for the target species under the conditions of use and was also deemed to be safe for the consumers of products derived from animals receiving the additive. Probiotics have been confirmed to improve nutrient digestion and absorption, ultimately enhancing chicken performance (Larsson *et al.*, 2012).

Growth promotion through immunomodulating activity

Ingestion of the probiotic *C. butyricum* induces beneficial effects in chickens, such as stimulation of the immune response, which is important for host health. *C. butyricum* has been widely studied for its immune activity through the activation of the immune system, regulation of immunity, and growth-promoting functions in the host (Huang *et al.*, 2019; Terada *et al.*, 2020; Zhao *et al.*, 2020a). The immunomodulatory effects of *C. butyricum* are mainly related to its defensive actions by adhering to the gut wall and stimulating immune cells, thereby promoting the release of cytokines to regulate immune responses. Huang *et al.* (2019) showed that the supplementation of *C. butyricum* increased the relative expression levels of *TLR2*, *IL-10,* and *TNF-α* in chickens with necrotic enteritis. This immune regulatory effect was also accompanied by an improvement in growth performance. Zhang *et al.* (2014) found that *C. butyricum* could alleviate *E. coli* K88-challenged immune stress in broilers, which might have contributed to a reduction in mortality and significantly greater ($P < 0.05$) body weight. Similarly, Yang *et al.* (2020) reported that *C. butyricum* restored the expression of intestinal cytokines (such as TNF-α, IL-10, IL-6, and TGF-β1), improved intestinal barrier function, and reduced the serum IgA and endotoxin contents, thereby exerting immunostimulatory and immunotherapeutic effects on broiler chickens infected with necrotic enteritis, ultimately resulting in beneficial effects on growth performance, including a remarkable increase in the average daily gain and feed conversion ratio ($P < 0.01$). Moreover, *C. butyricum* can stimulate immune responses due to its synergistic effects with other probiotics. A dietary combination of *C. butyricum* and *Saccharomyces cerevisiae* improved body weight gain, increased hemagglutination inhibition titers for the Newcastle disease virus, and enhanced the immune status of broiler chicks (Abdel-Latif *et al.*, 2018). Cai *et al.* (2022) showed that the improvement of immune responses following treatment of a combination of *C. butyricum* and coccidiosis vaccine significantly improved the overall performance of broiler chickens. Another probiotic combination of *C. butyricum*, *B. subtilis*, and *Bacillus licheniformis* significantly improved the final and average body weights, which may have been due to an improvement in the serum immune response (Zeng et al., 2021). Han *et al.* (2018) investigated the effects of *C. butyricum* combined with *Lactobacillus butyricum* and found that serum immunoglobulin and volatile fatty acid levels increased in *C. butyricum*-supplemented broilers; however, the combined additive showed no obvious effects on the growth performance of broilers. Therefore, future studies on the effects of *C. butyricum* supplementation in chickens should consider various factors such as chicken breed, levels of *C. butyricum* addition, and synergistic effects.

Growth promotion through antioxidative activity

Studies have shown that *C. butyricum* regulates the antioxidant system in chickens (Liao *et al.*, 2015; Yang *et al.*, 2022a). Broiler meat is popular globally owing to its low fat content and high concentrations of polyunsaturated fatty acids (Parra *et al.*, 2010; Kamboh and Zhu, 2013; Disetlhe *et al.*, 2019; Ma *et al.*, 2021). However, polyunsaturated fatty acids are highly susceptible to oxidative stress (Arshad *et al.*, 2013; Yang *et al.*, 2022c). Previous studies have found that dietary probiotics can increase the polyunsaturated fatty acid concentration as a protective mechanism, which is beneficial for meat quality (Liao *et al.*, 2015; Cramer *et al.*, 2018; Yang *et al.*, 2022a). Liao *et al.* (2015) found that dietary supplementation with *C. butyricum* relieved oxidative stress by increasing the superoxide dismutase activity and decreasing the malondialdehyde (MDA) content, thereby improving the meat quality and fatty acid composition of broilers. Another study confirmed that *C. butyricum* enhanced broiler meat quality and fatty acid composition, accompanied by improvements in the antioxidant capacity, such as an increase in the serum total antioxidant capacity, total superoxide dismutase, and catalase levels, and a reduction in MDA content, as well as its negative linear effects on cooking loss and shearing force (Yang *et al.*, 2022a). Similarly, *C. butyricum* supplementation had beneficial effects on the growth performance and egg quality of laying hens, as it reduced the average daily feed intake; increased the feed conversion, eggshell strength, and crude protein proportion; and reduced the serum MDA content and reactive oxygen species levels in the ileum and cecum (Xiang *et al.*, 2019). Thus, the antioxidative effects of *C. butyricum* in laying hens are beneficial for laying performance and egg quality. This evidence reveals that the antioxidant capacity of *C. butyricum* is closely associated with meat and egg quality, although investigations into the underlying mechanisms are limited and unclear, thus necessitating further studies.

Growth promotion through lipid metabolism regulation

Recent studies have described the role of lipid and fat metabolism in the health and performance of chickens. Thus, it is equally important to understand the influence of *C. butyricum* and its metabolites in these areas. The addition of *C. butyricum* reduced fat deposition and accelerated hepatic fatty acid oxidation by shaping the gut microbiota and bile acid profile of aged Hy-Line Brown laying hens (Wang *et al.*, 2020b). The combined use of *C. butyricum* Sx-01 and *Lactobacillus salivarius* C-1-3 reduced the amount of lipids by altering the gut microbiota in mice (Long *et al*., 2018). Dietary *C. butyricum* reduced breast muscle fatty acid oxidation in broilers (Zhao *et al.*, 2018) and increased the intramuscular fat content of the thigh muscles of broilers at 21 days (Zhao *et al.*, 2017). The metabolites of *C. butyricum* and combination with other probiotics also elicit positive effects on the growth performance of chickens. Zhang *et al.* (2016) reported that dietary supplementation with *C. butyricum* in broilers challenged with *E. coli* K88 increased amylase, protease, and lipase activities, consequently improving the body weight and average daily gain of broilers. In addition, Hossain *et al.* (2015) reported that dietary tri-strain probiotics (*B. subtilis*, *C. butyricum,* and *Lactobacillus acidophilus*) improved body weight gain and the feed conversion ratio by altering the gut microbiota, decreasing excreta ammonia gas emissions, and improving nutrient digestibility.

Collectively, intestinal microbes and probiotics act synergistically to support barrier function, improve growth performance, and maintain host health in chickens. Given the roles of *C. butyricum* in maintaining the intestinal barrier, gut microbes, and growth performance, this review provides theoretical support for the use of *C. butyricum* as a dietary probiotic additive in chicken nutrition.

Conclusions and Perspectives

Given the ban on antibiotics, the chicken industry urgently needs to identify green alternatives to antibiotics to improve the intestinal health of birds. *C. butyricum,* a well-known gram-positive obligate anaerobic bacillus with potent probiotic properties, could serve as a potential antibiotic substitute for modulating the gut health of chickens. Studies have shown that *C. butyricum* can increase the abundance of beneficial bacteria in the intestine, maintain intestinal homeostasis, protect the intestinal barrier, and inhibit the development of diseases caused by disorders of intestinal microbes, thereby improving growth performance in chickens. Although there have been several studies on the effects of *C. butyricum* on gut health to date, the microbiome-metabolomics approach has not been extensively utilized, especially in chickens. Advanced multi-omics analyses will provide valuable insights into the existing relationships between *C. butyricum*, gut microbes, intestinal barrier function, host health, and growth performance in chickens.

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Author Contributions

Min Liu, **Xikang Cao,** and **Xinyu Liu** summarized the relevant research, drafted the paper, and designed the figures; **Victoria Anthony Uyanga** was involved in planning, reviewing, and editing this review, especially language modification; **Hai Lin** contributed to the design and preparation of the review, funding acquisition, and review and editing of the paper. All the authors discussed the review and commented on the paper.

Conflicts of Interest

The authors declare no conflict of interest.

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