Research Advances in the Application of Clostridium butyricum in Livestock and Poultry Production (Postprint)

Authors: Zhao Minmeng, Shan Haoshu, Shen Yonghua, Zhang Cheng, Liu Long, Gong Daoqing

Date: 2018-12-24T00:00:00+00:00

Abstract

Clostridium butyricum possesses multiple physiological functions, including regulation of intestinal microecological balance, production of digestive enzymes and nutrients such as vitamins, enhancement of animal immunity, anti-apoptosis, and anti-tumor effects. Furthermore, Clostridium butyricum belongs to the anaerobic spore-forming bacilli, exhibiting favorable tolerance to high temperature and high pressure during feed processing as well as the gastrointestinal environment of animals. Therefore, Clostridium butyricum represents a suitable probiotic for supplementation in livestock and poultry production. This article summarizes the biological characteristics, physiological functions, effects on livestock and poultry production, and underlying mechanisms of action of Clostridium butyricum, providing a reference for its further research, development, and application.

Full Text

Research Progress on the Application of *Clostridium butyricum* in Livestock and Poultry Production

ZHAO Minmeng[1], SHAN Haoshu[2], SHEN Yonghua[3], ZHANG Cheng[3], LIU Long[1], GONG Daoqing[1]*

[1]College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
[2]Zhenjiang Animal Disease Prevention and Control Center, Zhenjiang 212009, China
[3]Dongtai Animal Husbandry and Veterinary Station, Dongtai 224200, China

Abstract: *Clostridium butyricum* possesses multiple physiological functions, including regulation of intestinal microecological balance, production of digestive enzymes and vitamins, enhancement of animal immunity, anti-apoptosis, and anti-tumor activity. As an obligate anaerobic spore-forming bacterium, it exhibits excellent tolerance to the high temperature and pressure conditions of feed processing as well as to the gastrointestinal environment of animals. Consequently, *C. butyricum* represents a promising probiotic candidate for livestock and poultry production. This review summarizes the biological characteristics, physiological functions, effects on livestock and poultry production, and underlying mechanisms of action of *C. butyricum*, providing a reference for its further research and development.

Keywords: *Clostridium butyricum*; probiotic; physiological function; livestock and poultry production

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1.1 Biological Characteristics of *Clostridium butyricum*

Taxonomically, *Clostridium butyricum* belongs to the phylum Firmicutes, class Clostridia, order Clostridiales, family Clostridiaceae, and genus *Clostridium*. This bacterium is a motile, spore-forming, obligate anaerobic bacillus with flagella. Like other bacilli, *C. butyricum* can exist in spore form, conferring strong resistance to environmental stresses. Research has demonstrated that *C. butyricum* tolerates the high-temperature and high-pressure conditions of feed processing effectively. Additionally, it shows robust resistance to the gastrointestinal environment, including artificial gastric juice, intestinal fluid, and bile salts. These characteristics make *C. butyricum* a highly resilient probiotic suitable for supplementation in livestock and poultry diets, ensuring its effective utilization by animals.

1.2 Physiological Functions of *Clostridium butyricum*

Clostridium butyricum and its metabolites exert numerous physiological functions that are beneficial for maintaining animal health and promoting growth. The intestinal tract of livestock and poultry develops a delicate microecological balance during maturation; disruption of this balance can lead to disease, compromised health, and reduced growth performance. First, *C. butyricum* promotes the growth of beneficial bacteria while inhibiting pathogens such as *Escherichia coli* and *Salmonella*, thereby protecting intestinal microecological balance. Second, butyric acid, a primary metabolite of *C. butyricum*, serves as the main energy source for intestinal epithelial cells and stimulates their proliferation, making it crucial for intestinal epithelial regeneration and repair. Third, *C. butyricum* produces various digestive enzymes in the animal gastrointestinal tract, including cellulase, lipase, and amylase, which improve feed utilization efficiency and mitigate the effects of certain anti-nutritional factors. The bacterium also synthesizes multiple B vitamins, which are important for improving

growth performance and maintaining animal health. Furthermore, studies have found that *C. butyricum* may enhance immunity, inhibit apoptosis, and exhibit anti-tumor effects. Further investigation into its physiological functions, mechanisms of action, and synergistic effects with other probiotics will provide a theoretical basis for product development and application.

2.1 Improvement of Growth Performance

Clostridium butyricum produces various digestive enzymes, amino acids, and vitamins that benefit animal health and promote nutrient digestion and absorption, thereby improving growth performance. Studies in broilers have shown that supplementation with *C. butyricum*-containing preparations improves growth performance by increasing average daily gain (ADG) and average daily feed intake (ADFI) from 1 to 42 days of age. Liao et al. reported that different supplementation levels of *C. butyricum* significantly increased ADG in broilers during 1-21 days of age, and at a dose of 5×10 CFU/kg, also significantly improved ADG during 22-42 days of age. This suggests that *C. butyricum* is particularly effective during early growth stages, while higher doses may be required for optimal effects during later stages. In Cherry Valley ducks, dietary supplementation with *C. butyricum* significantly increased ADG during 1-21 days of age and showed a tendency to improve ADG during 22-42 days of age. Similarly, studies in piglets demonstrated that dietary *C. butyricum* supplementation significantly increased ADFI and ADG in weaned piglets, improved feed conversion efficiency, and promoted growth. These findings indicate that *C. butyricum* supplementation can improve growth performance in broilers, ducks, and piglets, though the magnitude of effects varies. The mechanisms likely involve regulation of intestinal microecological balance, promotion of intestinal development, and improved feed utilization. However, some studies have reported no significant effects on growth performance, suggesting that the efficacy of *C. butyricum* depends heavily on animal species, production stage, strain characteristics, fermentation level, and supplementation dosage. Therefore, developing strain-specific preparations and determining optimal dosages for different livestock species and growth stages is essential. Moreover, as antibiotic use becomes increasingly restricted in modern intensive animal production, investigating the combined use of *C. butyricum* with other feed additives to address complex production environments and pathogen challenges represents a promising research direction.

2.2 Regulation of Intestinal Microecological Balance

In vitro studies have demonstrated that *Clostridium butyricum* significantly inhibits pathogenic bacteria including *E. coli*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Salmonella*, while promoting the growth of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. In broilers, dietary supplementation with *C. butyricum* inhibited the growth of *E. coli* and *Salmonella* in the cecum while promoting the proliferation of *Bifidobacterium* and *Lactobacillus*. Studies

in piglets also showed that *C. butyricum* improved intestinal flora structure, reduced intestinal pH, increased *Lactobacillus* populations, and decreased *E. coli* populations, effectively alleviating weaning stress. Both in vitro and in vivo experiments confirm that *C. butyricum* inhibits pathogen growth while promoting beneficial bacteria, thereby maintaining intestinal microecological balance. Several mechanisms may explain this effect. First, the primary metabolites of *C. butyricum*—butyric acid and acetic acid—are short-chain fatty acids that lower intestinal pH to inhibit harmful bacteria while promoting beneficial bacterial growth. Second, *C. butyricum* produces amylase that hydrolyzes dietary carbohydrates to form oligosaccharides, which are readily utilized by probiotics like *Lactobacillus* and *Bifidobacterium*. Wang et al. isolated a *C. butyricum* strain ZJU-F1 that secretes bacteriocin-like inhibitory proteins. Bacteriocins are antimicrobial substances produced by bacteria that may contribute to pathogen inhibition. Additionally, *C. butyricum* may inhibit pathogen colonization by occupying adhesion sites on intestinal epithelial cells, thereby preventing the attachment of transient pathogens.

2.3 Promotion of Intestinal Development and Barrier Function

The intestine is the primary site for nutrient digestion and absorption, with villus height, crypt depth, and the villus height-to-crypt depth ratio serving as important indicators of intestinal digestive function. Studies have shown that *Clostridium butyricum* supplementation significantly increased villus height and the villus height-to-crypt depth ratio in the duodenum, jejunum, and ileum of layer cockerels, improving intestinal morphology. Similarly, dietary *C. butyricum* supplementation significantly increased small intestinal villus height and decreased crypt depth in weaned piglets. These findings demonstrate that *C. butyricum* promotes intestinal development and improves digestive function, likely through its primary metabolite butyric acid, which serves as the main nutrient for intestinal epithelial cells and plays a vital role in epithelial regeneration and repair. Beyond digestion and absorption, the intestine functions as a congenital barrier critical for maintaining animal health. Research has shown that dietary supplementation with 5×10 CFU/kg *C. butyricum* significantly increased mRNA expression levels of tight junction proteins claudin-1 and ZO-2 in the jejunum and ileum of weaned piglets. Tight junctions between intestinal epithelial cells are essential for regulating intestinal barrier permeability and maintaining epithelial integrity, and accelerated expression of these proteins promotes maturation of barrier function. Yang et al. also found that *C. butyricum* could inhibit expression of pathogenic virulence proteins through direct cell contact, reducing colonization by harmful bacteria. Thus, *C. butyricum* enhances intestinal barrier function by promoting mucosal development to strengthen mechanical barriers and regulating microbial balance to improve microbial barriers.

2.4 Enhancement of Immunity and Reduction of Inflammation

Clostridium butyricum plays a regulatory role in activating and modulating immune function through multiple mechanisms. In Cherry Valley ducks, supplementation with 5×10 CFU/kg *C. butyricum* significantly increased thymus and spleen indices, promoting immune organ development, while also significantly elevating serum complement 3 (C3) and complement 4 (C4) levels at 42 days of age. In weaned piglets, dietary supplementation with 500 mg/kg *C. butyricum* significantly increased serum C3 content. C3 is the most abundant complement component in serum and plays a crucial role in both classical and alternative complement activation pathways, while C4 is a multifunctional 1-globulin involved in complement activation and viral neutralization. These results indicate that *C. butyricum* promotes immune organ development, activates the complement system, and enhances immunity, thereby benefiting animal health. Additionally, studies have shown that *C. butyricum* supplementation significantly increased serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) levels in broilers and ducks, with immunoglobulins providing antimicrobial and antiviral functions representing another mechanism for immune enhancement. Cell culture studies have revealed that *C. butyricum* promotes expression of the anti-inflammatory cytokine interleukin-10 (IL-10) while inhibiting expression of the pro-inflammatory cytokine interleukin-4 (IL-4). Animal experiments have further demonstrated that *C. butyricum* increases serum IL-10 content, significantly decreases pro-inflammatory cytokine IL-1 levels, and modulates inflammatory responses through regulation of nuclear factor-kappa B (NF- B). These findings indicate that *C. butyricum* enhances immune function and reduces inflammation by modulating cellular immunity and altering serum cytokine profiles. Wang et al. found that *C. butyricum* supplementation in piglets increased mRNA expression levels of Toll-like receptors 2 and 4 and the adaptor protein MyD88 in the ileum, with activation of this pathway inducing IL-10 secretion. This suggests that *C. butyricum* may also enhance immunity by suppressing intestinal inflammation. In summary, the immunomodulatory effects of *C. butyricum* are associated with promotion of immune organ development, activation of the complement system, regulation of immunoglobulin levels, modulation of serum cytokines, and suppression of intestinal inflammation.

2.5 Regulation of Lipid Metabolism and Improvement of Meat Quality

Clostridium butyricum also influences lipid metabolism and meat quality in animals. Dietary supplementation with 1×10 CFU/kg *C. butyricum* increased intramuscular fat content in broiler breast and thigh muscles by affecting lipogenic enzyme activity and lipid synthesis-related gene expression. Angiopoietin-like protein 4 (ANGPTL4) is a protein associated with lipid metabolism and obesity that reduces fat deposition by inhibiting lipoprotein lipase activity and promoting lipolysis and fatty acid oxidation. Research suggests that *C. butyricum* reduces lipogenesis by increasing ANGPTL4 gene expression and protein secre-

tion in Caco-2 cells and decreasing expression of lipogenic genes in HepG2 cells, with the cell wall components and butyric acid as the primary active fractions. Shang et al. reported that mice fed a high-fat diet supplemented with *C. butyricum* for 12 weeks showed 17% lower body weight than the high-fat control group, with reduced liver fat content and improved insulin sensitivity, indicating that *C. butyricum* alleviates high-fat diet-induced obesity and fatty liver, though its effects vary among organs. In intensive animal production, increased dietary energy levels, high feed intake, reduced activity, and environmental stress have markedly increased the incidence of fatty liver disease, leading to decreased performance and significant economic losses. As a lipid metabolism regulator, *C. butyricum* may have therapeutic value for fatty liver disease in livestock and poultry. In poultry, muscle fat and fatty acid composition are strongly correlated with meat quality traits including tenderness, shear force, pH, water-holding capacity, and flavor, with increased intramuscular fat improving flavor and tenderness. Dietary supplementation with 1×10 CFU/kg *C. butyricum* significantly increased intramuscular fat content in thigh muscle at 42 days of age but not at 21 days of age. Liao et al. found that *C. butyricum* supplementation significantly increased polyunsaturated fatty acid (PUFA) content and the PUFA to saturated fatty acid (SFA) ratio in broiler breast muscle at 42 days of age, with PUFAs contributing to characteristic flavor compounds and improved meat quality. Additionally, *C. butyricum* supplementation significantly reduced muscle shear force in broilers. These findings demonstrate that *C. butyricum* improves meat quality, likely through effects on intramuscular fat deposition and PUFA content, though the specific mechanisms require further investigation.

2.6 Enhancement of Antioxidant Capacity

Recent studies have increasingly demonstrated the antioxidant functions of probiotics including *Clostridium butyricum*. This bacterium can produce total superoxide dismutase (T-SOD) and NADH/NADPH peroxidase to scavenge reactive oxygen species and enhance antioxidant capacity. Dietary *C. butyricum* supplementation significantly increased serum glutathione peroxidase (GSH-Px) and T-SOD activities in 42-day-old broilers. Furthermore, supplementation significantly improved antioxidant capacity in the liver and duodenal, jejunal, and ileal mucosa of broilers at both 21 and 42 days of age. Liao Xiudong found that *C. butyricum* alleviated corticosterone-induced oxidative stress by restoring hepatic glutathione S-transferase (GST) activity and glutathione (GSH) content while significantly reducing malondialdehyde (MDA) levels in broiler liver. These results indicate that *C. butyricum* enhances animal antioxidant capacity, possibly through production of digestive enzymes, butyric acid, and hydrogen gas (H), which affect antioxidant enzyme activity and reduce reactive oxygen species. The antioxidant effects may also be related to inhibition of pathogen growth, thereby reducing inflammation and associated oxidative stress.

3 Summary

In summary, *Clostridium butyricum* exhibits numerous physiological functions that improve growth performance, enhance animal health and disease resistance, and improve product quality in livestock and poultry. Its excellent tolerance to feed processing conditions and the gastrointestinal environment further supports its application as a probiotic feed additive. However, the mechanisms of action are complex and not yet fully understood, and exploring additional physiological functions and mechanisms remains a research priority. For example, the protective effects of *C. butyricum* against liver injury in non-alcoholic fatty liver disease suggest potential therapeutic applications for animal diseases that warrant investigation. Additionally, current research has focused primarily on broilers, ducks, and weaned piglets; exploring its application in other livestock such as laying hens and geese represents a feasible direction for future research.

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