Biological Functions of Lactic Acid Bacteria and Their Applications in Sow and Piglet Production: Postprint

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Abstract

Lactic acid bacteria are common probiotics that play important roles in maintaining animal intestinal microecological balance, promoting growth, and enhancing intestinal immunity. This article reviews the tolerance of lactic acid bacteria for survival in the gastrointestinal tract, their biological functions, and their effects on piglets and sows, and summarizes potential factors contributing to inconsistent application efficacy.

Full Text

Biological Functions of Lactobacillus and Its Application in Sow and Piglet Production

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Abstract: Lactobacillus is a common probiotic that plays important roles in maintaining intestinal microecological balance, promoting growth, and enhancing gut immunity. This paper reviews the gastrointestinal tolerance and biological functions of Lactobacillus, its effects on suckling piglets, weaned piglets, and sows, and summarizes the potential factors causing inconsistent application outcomes.

Keywords: Lactobacillus; tolerance; biological function; pigs

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In modern swine production systems, factors such as oxidative stress, impaired intestinal barrier function, and low immunity often lead to slow growth, poor feed utilization, and diarrhea, severely affecting the economic efficiency of pig enterprises. Although antibiotic supplementation can ameliorate these problems, long-term abuse inevitably causes antibiotic residues and bacterial resistance issues. As feed additives, antibiotics are gradually being restricted or banned, making probiotics promising antibiotic alternatives. Lactobacillus, a type of probiotic, is a Gram-positive anaerobic or facultative anaerobic microorganism that primarily produces lactic acid through fermentation. It exerts functional effects by improving intestinal microbial balance, enhancing antioxidant defense systems, regulating intestinal mucosal immunity, and maintaining intestinal barrier function, though its practical application effects are not always obvious. This review summarizes the gastrointestinal tolerance, biological functions of Lactobacillus, and its effects on suckling piglets, weaned piglets, and sows, and outlines potential factors causing inconsistent application outcomes.

1 Gastrointestinal Tolerance of Lactobacillus

Probiotics must survive in gastric acid for 4 hours or longer before reaching the intestine. Therefore, tolerance to acid and bile salts is a crucial criterion for selecting probiotic strains. Acid tolerance is determined by Lactobacillus' s ability to maintain intracellular pH [1], preserve cell membrane function [2], or induce stress response proteins [3]. Bile salt tolerance is determined by active efflux of bile acids/salts, bile salt hydrolysis [4], and alterations to the cell membrane [5]. Studies have shown that *Lactobacillus casei* induces expression of proteins involved in carbohydrate metabolism to provide energy for resisting acid stress [6]. *Lactobacillus plantarum* ZlP001 isolated from piglet intestines exhibited tolerance rates of 85.3%, 61.4%, and 9.4% when inoculated into media containing 0.1%, 0.3%, and 0.5% bile acids, respectively, and demonstrated tolerance to simulated gastric and intestinal fluids [7]. Therefore, screening strains with gastrointestinal survival tolerance *in vitro* facilitates their growth and reproduction in the animal intestine.

2.1 Maintaining Intestinal Microecological Balance

The ability of pathogenic bacteria to adhere to epithelial cell brush borders is key to host infection. Studies have shown that Lactobacillus achieves 80% adhesion in human colon adenocarcinoma (Caco-2) cell lines [8]. The exopolysaccharide produced by *Lactobacillus paracasei* BGSJ2-8 in the intestine can adhere to intestinal epithelial cells and reduce *E. coli* binding to Caco-2 cells [9]. When cocultured with *Staphylococcus aureus*, *L. plantarum* inhibits the latter' s growth by 87% [10]. Lactobacillus adhesion is a prerequisite for colonization and proliferation in the animal intestine, depending on bacterial structure, secretions, and corresponding receptors on the host cell surface. The collagen-binding protein of *Lactobacillus fermentum* 3872 binds to type I collagen on intestinal epithelial cells, which has been identified as an adhesin involved in binding [11]. Lactobacillus colonization in the intestine likely occurs through lipoteichoic acid, peptidoglycan, and exopolysaccharides on the bacterial surface acting as adhesins that bind to specific pattern recognition receptors on the intestinal mucosa [12], which helps alleviate exclusion effects from intestinal peristalsis.

Intestinal microorganisms compete for attachment sites on epithelial cell brush borders. Highly adhesive beneficial bacteria can prevent pathogen adhesion and invasion. *Lactobacillus delbrueckii*, *Lactobacillus rhamnosus* GG, and *L. fermentum* I5007 can inhibit adhesion and colonization of pathogenic bacteria such as *E. coli*, *Salmonella*, and *Candida* in the host intestine through exclusion, competition, or displacement [13-15]. Research has found that *L. rhamnosus* GG alleviates epithelial damage by reducing *Candida* adhesion to mucosa and consuming glucose [15], indicating that Lactobacillus can form a biological barrier by preferentially utilizing intestinal nutrients and competing for adhesion sites, causing pathogens to starve and be expelled. Studies have shown that bacterial metabolites produced by Lactobacillus inhibit Gram-positive bacterial growth [16]; *Lactobacillus johnsonii* NCC533 produces hydrogen peroxide *in vivo*, effectively inhibiting *Salmonella* growth [17]; and *Lactobacillus reuteri* I5007 changes intestinal pH through lactic acid metabolism, hindering pathogen colonization [18]. These findings demonstrate that Lactobacillus can inhibit pathogen growth and reproduction. Therefore, extensive Lactobacillus colonization and proliferation in the intestine, along with its metabolic products, affects colonization of pathogenic microorganisms, thereby maintaining intestinal microecological balance.

2.2 Antioxidant Defense Function

Under normal conditions, free radical production and clearance in animals maintain a dynamic balance. Once disrupted, excessive free radicals accumulate and damage biological macromolecules such as DNA and lipids, causing oxidative stress damage. Most researchers currently believe that Lactobacillus antioxidant mechanisms mainly include free radical scavenging, metal ion chelation, reducing capacity, lipid oxidation inhibition, and enhanced antioxidant enzyme activity. *In vitro* studies have shown that *L. plantarum* ZLP001 can scavenge superoxide and hydroxyl radicals [19]; *L. fermentum* ME-3 improves overall antioxidant levels by reducing metal ions and lipid peroxidation [20]; and feeding *L. plantarum* C88 to D-galactose-induced oxidative stress mice increased superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and total antioxidant capacity (T-AOC) in liver and serum while reducing malondialdehyde (MDA) content in liver [21]. Therefore, dietary Lactobacillus supplementation can maintain the dynamic balance of free radicals in animals and alleviate oxidative stress damage.

2.3 Intestinal Barrier Function and Immunity

Establishment of intestinal microbiota is crucial for animal intestinal immune systems. Exogenous Lactobacillus positively affects intestinal structure and barrier integrity. Reports indicate that *L. reuteri* D8, as an exogenous factor, has biological effects on intestinal stem cells. *L. reuteri* D8 induces lamina propria lymphocytes to secrete interleukin (IL)-22 by activating the aryl hydrocarbon receptor, which subsequently activates signal transducer and activator of transcription 3 phosphorylation to accelerate intestinal stem cell regeneration and restore tumor necrosis factor (TNF)- -damaged intestinal epithelial structure [22]. It also upregulates mRNA expression of zonula occludens-1 (ZO-1), occludin, and claudins, stabilizing tight junction protein structure and distribution to improve intestinal barrier function [23]. Studies have also suggested that Lactobacillus may regulate intestinal immunity by affecting other microbial communities or mediating metabolites and self-produced immune promoters after entering the intestine [24-26]. *L. rhamnosus* induces IL-10 synthesis, which inhibits secretion of pro-inflammatory factors such as TNF- and interferon (IFN)- to regulate immune function [24]; *Lactobacillus salivarius* LA307 alleviates colonic inflammation in mice, while *Lactobacillus helveticus* PI5 maintains intestinal epithelial barrier integrity [26]. Additionally, there is an association between *L. reuteri* and the generation of tolerance-promoting immune cells. When *L. reuteri* was transplanted into germ-free mice, dietary tryptophan supplementation positively affected immune cell production [27], indicating that certain amino acids and intestinal microorganisms are not independent. The interaction between certain amino acids and intestinal microbiota is currently receiving significant research attention, providing new insights for intestinal mucosal immune mechanisms and intervention strategies.

3.1 Application in Suckling Piglet Production

Newborn piglet intestines are essentially sterile, with *Bifidobacterium*, *Lactobacillus*, *E. coli*, and *Enterococcus* colonizing within 24 hours after birth. Bacterial richness and diversity are positively correlated with age. Therefore, early intervention in piglet intestinal microbiota establishment is crucial for intestinal health. Studies have shown that exogenous *L. fermentum* in early piglet life increases villus height and crypt depth in the duodenum and jejunum, upregulates ZO-1 and occludin mRNA expression in the jejunum, maintains intestinal morphology and mucosal permeability, improves barrier function, and effectively alleviates diarrhea in newborn piglets induced by enterotoxigenic *E. coli* stress [28]. Liu et al. [13] found that on day 14 of the trial, piglets showed reduced *Clostridium* counts in the colon, increased villus height in the jejunum, increased butyrate and fatty acid concentrations in the colon, and decreased IL-1 mRNA expression in the ileum. After feeding piglets with *L. fermentum* I5007, the proportion of CD4+ T cell subsets in blood and INF- content in the ileum increased, while diarrhea rate decreased [29]. Zhang et al. [30] reported that *L. salivarius* significantly upregulated -defensin-2 secretion in suckling piglets, enhancing intestinal chemical barrier function. Due to immature digestive and immune systems in young piglets, their intestines lack sufficient acid to aid digestion and are susceptible to pathogen infection causing diarrhea. Lactobacillus promotes metabolism and nutrient absorption while improving intestinal health [31]. Studies have shown that oral administration of *L. casei* to suckling piglets increased protease activity in the stomach, duodenum, and colon, as well as plasma immunoglobulin A (IgA) content [32]. Therefore, exogenous Lactobacillus can effectively enhance gastrointestinal function in suckling piglets, likely by inducing optimal microbial community composition, improving barrier function, enhancing immunity, and preventing pathogen infection.

3.2 Application in Weaned Piglet Production

In recent years, Lactobacillus has received widespread attention for improving growth performance and intestinal health in weaned piglets. During weaning stress, piglets typically experience reduced feed intake, decreased intestinal digestive enzyme activity, and diarrhea, primarily due to disrupted intestinal microecological balance [33]. Zhang et al. [34] found that adding 0.75% porcine-derived *Lactobacillus reuteri* as a feed additive increased average daily gain by 20.07% and reduced feed-to-gain ratio by 14.90% in piglets. Wang et al. [35] also demonstrated that dietary supplementation with 1×10 CFU/d of *L. plantarum* ZJ316 increased average daily gain by 20.45% and improved feed conversion rate by 21.07%. However, some reports indicate that exogenous Lactobacillus has no significant effect on piglet growth performance [36]. Lactobacillus exhibits strain specificity, which may affect bacterial-host interactions. Non-endogenous strains for the host may have low survival rates in the intestine, fail to become dominant flora, and thus have limited probiotic effects. Studies have shown that Lactobacillus can replace antibiotics in piglets [37], and *L. fermentum* I5007 alleviates weaning stress syndrome by increasing protein levels related to energy metabolism, lipid metabolism, protein synthesis, and immune response [38]. After feeding weaned piglets with different Lactobacillus strains, serum immunoglobulin M (IgM) and IFN- content increased [39]; secretory immunoglobulin A (sIgA) secretion in jejunal and ileal mucosa increased [40]; IgA secretion was promoted while ileal IL-8 secretion decreased, alleviating inflammation caused by *Salmonella* infection [41]; and ZO-1, occludin, and claudins mRNA expression was upregulated [23], stabilizing tight junction protein structure and distribution, reducing intestinal mucosal permeability, and improving intestinal health. Additionally, weaning stress is associated with oxidative stress, which is considered a major factor affecting intestinal health and function. Studies have shown that *L. plantarum* ZLP001 increased serum SOD, GSH-Px, and catalase (CAT) activities while reducing serum MDA content in weaned piglets [19], improving antioxidant function. Therefore, dietary Lactobacillus supplementation can improve growth performance, immunity, and antioxidant capacity in weaned piglets, thereby alleviating weaning stress.

3.3 Application in Sow Production

Changes in intestinal microbiota structure participate in host metabolic regulation, including oxidative stress and inflammatory responses. Studies have shown that high-yielding sows experience DNA oxidative damage and decreased blood antioxidant content during late gestation and lactation [42]. Ren et al. [43] found that dietary supplementation with a Lactobacillus-yeast composite significantly increased plasma total cholesterol, triglyceride, and high-density lipoprotein cholesterol content in sows, reduced plasma MDA content, and increased litter birth weight, thereby improving reproductive performance, plasma lipid metabolism, and antioxidant capacity. Intestinal microbiota in sows becomes disordered to varying degrees during late gestation, which may increase reactive oxygen species in the intestinal lumen and damage colonic epithelial cell DNA. However, some commensal bacteria such as certain Lactobacillus species have antioxidant effects and can inhibit reactive oxygen species production. *Lactobacillus paracasei* Fn032, *L. rhamnosus* GG, and others can inhibit free radical production during colonic chyme fermentation and suppress *E. coli* growth [44]. High neonatal piglet mortality is typically associated with diarrhea caused by *E. coli*, *Salmonella*, rotavirus, and coronavirus infections. Piglet immunity against infection is primarily established through immunoglobulins and cytokines in colostrum. Cytokines have been shown to exert local effects on intestinal mucosa and penetrate the circulatory system, affecting colonization of gastrointestinal commensal microorganisms in piglets [45]. Studies have shown that feeding probiotics affects sow intestinal microbiota [46] and colostrum composition [47]. Wang et al. [48] reported that dietary Lactobacillus supplementation during gestation significantly increased piglet birth and weaning litter weights and serum immunoglobulin G (IgG) content, while improving piglet intestinal microbial diversity [49]. Therefore, dietary Lactobacillus supplementation in late gestation may alleviate metabolic disorders in sows and affect neonatal piglet passive immunity and intestinal microbiota composition by altering sow gut microbiota and colostrum composition during gestation, thereby promoting piglet intestinal health.

4 Summary

In summary, exogenous Lactobacillus functions and applications in sow and piglet production mainly occur through two pathways: 1) altering sow gestational intestinal microbiota and colostrum composition to affect neonatal piglet passive immunity and intestinal microbiota structure, promoting piglet intestinal health; and improving antioxidant capacity, intestinal barrier function, and immunity in weaned piglets to alleviate weaning stress; 2) alleviating oxidative stress and inflammatory responses in sows during gestation. However, the practical application effects of Lactobacillus and other probiotics are often unsatisfactory, primarily due to the following factors: 1) Lactobacillus production requires extremely high technical conditions and is difficult to cultivate, resulting in limited practical feed-grade Lactobacillus products on the market. 2) Natural loss and inactivation rates are very high during actual storage. Additionally, during pellet feed production, unprotected Lactobacillus is basically eliminated after processing at temperatures above 80°C. 3) Application effects are closely related to the quantity of strains used; low quantities in the intestine cannot form dominant flora and thus have limited probiotic effects. There are currently no unified regulations regarding the required microbial quantities for specific livestock species. Therefore, the key to effective feed-grade Lactobacillus additives lies in their effective content, encapsulation, heat resistance, storage stability, and pelleting resistance, as well as *in vitro* screening for gastrointestinal survival tolerance. Only by considering these factors can better application effects be achieved. Furthermore, potential concerns when applying live Lactobacillus to feed include pathogenicity, possibility of antibiotic resistance gene transfer, and uncontrollable reproduction and mutation.

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Note: Figure translations are in progress. See original paper for figures.