Effects of Clostridium butyricum on Growth Performance, Intestinal Structure, and Immune Function in Weaned Piglets: Postprint

Authors: Zheng Youxiu, Wang Chao, Zou Xiaoting, Wang Li, Lu Jianjun

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Abstract

This experiment was conducted to investigate the effects of dietary Clostridium butyricum supplementation on growth performance, intestinal structure, and immune function in weaned piglets. A total of 360 healthy "Duroc × Landrace × Yorkshire" crossbred piglets at (25$±1)*daysofagewithabodyweightof*(6.24±$0.32) kg were randomly allocated to 5 groups with 4 replicates of 18 piglets each. The control group was fed a basal diet, while the treatment groups were fed the basal diet supplemented with 250, 500, 1,000, and 2,000 mg/kg Clostridium butyricum, respectively. The experimental period lasted 30 days. The results showed that: 1) The average daily feed intake (ADFI) of weaned piglets in the 250 and 1,000 mg/kg Clostridium butyricum supplementation groups was significantly lower than that of the control group (P<0.05); the diarrhea rate in the 250 and 500 mg/kg Clostridium butyricum supplementation groups was significantly reduced by 40.99% and 44.32% compared with the control group (P<0.05), respectively. 2) The jejunal villus height of weaned piglets in the Clostridium butyricum supplementation groups was significantly increased by 32.23%, 35.71%, 33.59%, and 47.36% compared with the control group (P<0.05), and the jejunal villus height/crypt depth ratio was increased by 47.24% (P<0.01), 48.03% (P<0.01), 19.69% (P<0.05), and 22.83% (P<0.05), respectively. 3) Dietary Clostridium butyricum supplementation significantly increased the serum immunoglobulin A (IgA) and immunoglobulin G (IgG) contents in weaned piglets (P<0.05), while there were no significant differences in serum immunoglobulin M (IgM), complement 3 (C3), and complement 4 (C4) contents among all groups (P>0.05). These results indicate that dietary supplementation with 250–500 mg/kg Clostridium butyricum for 25- to 55-day-old weaned piglets can improve intestinal structure and enhance immune function.

Full Text

Effects of Clostridium butyricum on Growth Performance, Intestinal Structure and Immune Function of Weaned Piglets

ZHENG Youxiu[1], WANG Chao[2], ZOU Xiaoting[1], WANG Li[3], LU Jianjun[1]*

[1]Key Laboratory of Feed and Animal Nutrition of Zhejiang Province, Key Laboratory of Animal Nutrition and Feed in East China, Ministry of Agriculture, Key Laboratory of Animal Molecular Nutrition, Ministry of Education, College of Animal Sciences, Zhejiang University, Hangzhou 310058, China

[2]College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

[3]Hubei Green Snow Biological Industry Co., Ltd, Xianning 437110, China

Abstract

This experiment was conducted to investigate the effects of dietary Clostridium butyricum supplementation on growth performance, intestinal structure, and immune function of weaned piglets. A total of 360 healthy "Duroc × Landrace × Yorkshire" hybrid piglets at (25$±1)*daysofagewithaninitialbodyweightof*(6.24±$0.32) kg were randomly allocated into 5 groups with 4 replicates per group and 18 piglets per replicate. Piglets in the control group were fed a basal diet, while those in the experimental groups were fed the basal diet supplemented with 250, 500, 1,000, and 2,000 mg/kg of Clostridium butyricum, respectively. The experiment lasted for 30 days. The results showed that: 1) The average daily feed intake (ADFI) of piglets in the 250 and 1,000 mg/kg Clostridium butyricum groups was significantly lower than that in the control group (P<0.05). The diarrhea rate in the 250 and 500 mg/kg Clostridium butyricum groups was significantly reduced by 40.99% and 44.32% compared with the control group (P<0.05), respectively. 2) The jejunal villus height in Clostridium butyricum groups was significantly increased by 32.23%, 35.71%, 33.59%, and 47.36% compared with the control group (P<0.05), respectively. The jejunal villus height to crypt depth ratio was significantly increased by 47.24% (P<0.01), 48.03% (P<0.01), 19.69% (P<0.05), and 22.83% (P<0.05), respectively. 3) Dietary Clostridium butyricum supplementation significantly increased serum immunoglobulin A (IgA) and immunoglobulin G (IgG) contents in weaned piglets (P<0.05), while no significant differences were observed in serum immunoglobulin M (IgM), complement 3 (C3), and complement 4 (C4) contents among all groups (P>0.05). These results indicate that dietary supplementation of 250-500 mg/kg Clostridium butyricum can improve intestinal structure and enhance immune function in weaned piglets aged 25-55 days.

Keywords: Clostridium butyricum; weaned piglets; growth performance; intestinal structure; immunity

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Weaning is an essential phase in pig production. During weaning, piglets face nutritional, physiological, environmental, microbial, and immune stresses [1], which lead to intestinal dysfunction, reduced feed intake, diarrhea [2], and villus atrophy in the small intestine, affecting nutrient digestion and absorption [3]. Studies have shown that antibiotic supplementation in piglet diets can alleviate weaning stress to some extent [4]; however, long-term antibiotic use poses negative impacts on the ecological environment, livestock, and even human health [5-6]. Probiotics are novel feed additives that inhibit pathogen adhesion and proliferation, maintain microecological balance in the animal gastrointestinal tract, and produce beneficial metabolites that serve as nutrient sources for the host or aid in digestion and absorption. Due to their safety, efficacy, and pollution-free characteristics, probiotics have been gradually applied in medical, health care, food, livestock, and aquaculture industries [7].

Clostridium butyricum, also known as butyric acid bacteria, is a probiotic preparation of Gram-positive anaerobic bacteria with spore structure, enabling it to withstand high temperature and pressure during feed processing [8] and exhibit strong tolerance to artificial gastric juice, intestinal fluid, and bile salts [9]. As a normal flora in human and animal intestines, Clostridium butyricum produces various beneficial substances such as short-chain fatty acids, amino acids, and enzymes, promoting growth and enhancing immunity. Its primary metabolite, butyric acid, serves as a nutrient for intestinal epithelial cell regeneration and repair [10]. Based on its probiotic functions and feasibility as a feed additive, Clostridium butyricum has attracted widespread attention from researchers and practitioners and has been gradually applied in livestock production to improve performance, intestinal environment, structure, and immune function [11-13]. Recent studies have reported its application in pig production, but the effects have been inconsistent [14-16], and the mechanisms remain unclear, necessitating further research. This experiment investigated the effects of different dietary levels of Clostridium butyricum on growth performance, intestinal structure, and immune function of weaned piglets to provide a basis for its practical application.

1.1 Experimental Material

Clostridium butyricum was provided by Hubei Green Snow Biological Industry Co., Ltd, with a viable count of 2.0×10^{8} CFU/g.

1.2 Experimental Design

A total of 360 healthy "Duroc × Landrace × Yorkshire" hybrid piglets at (25 $\pm$ 1) days of age with an initial body weight of (6.24 $\pm$ 0.32) kg were randomly divided into 5 groups with 4 replicates per group and 18 piglets per replicate.

The control group was fed a corn-soybean meal basal diet without Clostridium butyricum, while the experimental groups were fed the basal diet supplemented with 250, 500, 1,000, and 2,000 mg/kg Clostridium butyricum, respectively. The basal diet was formulated according to the nutrient requirements of NRC (2012) and the nursery pre-starter formula of Jiahao Agriculture Co., Ltd. The composition and nutrient levels are shown in Table 1 . All diets were provided in powder form. The experimental period lasted for 30 days.

1.3 Feeding Management

The experiment was conducted in the nursery facility of Jiahao Agriculture Co., Ltd in Xiaoshan District, Hangzhou City, Zhejiang Province, using slatted floor nursery equipment. Each replicate of each group was housed in different nursery pens within the same nursery building to minimize environmental differences among groups. Feed was provided at 07:00, 14:00, and 21:00 daily, with feeding amounts adjusted to ensure slight residual feed in the troughs. Piglets had ad libitum access to feed and water throughout the experiment. Preventive vaccination and management followed conventional practices.

On the final day of the experiment, piglets were fasted for 12 h with free access to water. Following the principle of selecting at least one piglet per replicate, 6 piglets were randomly selected from each group (30 piglets total). After recording live weight before slaughter, piglets were euthanized by exsanguination via carotid artery. Blood samples were collected, and tissues from duodenum, jejunum, ileum, liver, and spleen were harvested.

1.4 Measurement Indicators

1.4.1 Growth Performance

At the beginning and end of the experiment, piglets in each replicate were weighed after fasting to calculate average daily gain (ADG). Daily feed intake was recorded for each replicate to calculate average daily feed intake (ADFI). Feed to gain ratio (F/G) was calculated based on ADG and ADFI. Diarrhea incidence was observed daily to calculate diarrhea rate. Mortality was recorded at the end of the experiment to calculate mortality rate.

F/G = Total feed consumption (kg) / Total piglet weight gain (kg);
Diarrhea rate (%) = [Number of piglets with diarrhea during experiment / (Total number of piglets × Experimental days)] × 100;
Mortality rate (%) = (Number of dead or culled piglets during experiment / Total number of piglets) × 100.

1.4.2 Intestinal Structure

Approximately 1 cm segments from the middle of duodenum, jejunum, and ileum were gently rinsed in 0.9% NaCl solution to remove intestinal contents, then fixed in 4% paraformaldehyde for at least 48 h. After washing, gradient alcohol dehydration, xylene clearing, and paraffin embedding, 5 m continuous sections were prepared and stained with routine hematoxylin-eosin (HE). Sections were observed under Nikon eclipse 80i confocal fluorescence microscope, and images were captured and measured using NIS-Elements BR 3.2 software. For each slice, 10 intact villi and crypts were selected for measurement, and the average values were calculated.

1.4.3 Immune Function

After slaughter, liver and spleen were isolated and weighed to calculate immune organ index.
Immune organ index (g/kg) = Fresh immune organ weight (g) / Live weight before slaughter (kg).

Blood samples were collected and allowed to clot. After serum separation, supernatant was collected and centrifuged at 3,500 r/min for 15 min. The supernatant was aliquoted and stored at -80°C. Serum complement 3 (C3), complement 4 (C4), immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Experimental data were initially processed using Excel 2003 and analyzed using SPSS 20.0 software via one-way ANOVA. Data were expressed as "mean ± standard deviation." Turkey' s multiple comparison test was used for significance testing among groups, with $P<0.05$ considered statistically significant.

2.1 Effects of Clostridium butyricum on Growth Performance of Weaned Piglets

As shown in Table 2 , dietary Clostridium butyricum supplementation had no significant effect on ADG and F/G of weaned piglets ($P>0.05$). The ADFI in 250 and 1,000 mg/kg Clostridium butyricum groups was significantly lower than that in the control group ($P<0.05$). The diarrhea rate in 250 and 500 mg/kg Clostridium butyricum groups was significantly reduced by 40.99% and 44.32% compared with the control group ($P<0.05$), respectively. As shown in Figure 1 [Figure 1: see original paper], dietary supplementation with 250, 500, and 2,000 mg/kg Clostridium butyricum reduced mortality by 67.11%, 49.94%, and 17.77%, respectively, but the differences were not statistically significant ($P>0.05$).

2.2 Effects of Clostridium butyricum on Intestinal Structure of Weaned Piglets

As shown in Table 3 , dietary Clostridium butyricum supplementation had no significant effect on villus height, villus height to crypt depth ratio in duodenum and ileum, or crypt depth in jejunum and ileum (P>0.05). Jejunal villus height in Clostridium butyricum groups was significantly increased by 32.23%, 35.71%, 33.59%, and 47.36% compared with the control group (P<0.05), respectively. The jejunal villus height to crypt depth ratio was significantly increased by 47.24% (P<0.01), 48.03% (P<0.01), 19.69% (P<0.05), and 22.83% (P<0.05), respectively. The duodenal crypt depth in the 500 mg/kg Clostridium butyricum group was significantly lower than that in the 250 and 2,000 mg/kg groups (P<0.05).

2.3 Effects of Clostridium butyricum on Immune Function of Weaned Piglets

No abnormalities were observed in the immune organs of slaughtered piglets. As shown in Figure 2 [Figure 2: see original paper], there were no significant differences in liver index and spleen index among all groups (P>0.05). As shown in Table 4 , serum IgA content in Clostridium butyricum groups was significantly increased by 112.11%, 112.32%, 165.55%, and 139.67% compared with the control group (P<0.05), respectively. Serum IgG content was significantly increased by 61.27%, 46.43%, 59.59%, and 43.33% (P<0.05), respectively. No significant differences were observed in serum IgM, C3, and C4 contents among all groups (P>0.05).

3 Discussion

3.1 Effects of Clostridium butyricum on Growth Performance of Weaned Piglets

Clostridium butyricum metabolism produces amylase, vitamins, and amino acids, which promote fat and protein digestion and absorption [17]. The present results showed that dietary Clostridium butyricum supplementation tended to increase ADG and decrease F/G, consistent with the findings of Pang et al. [15]. However, other studies have reported that Clostridium butyricum significantly increased ADG in weaned piglets [14,18]. These inconsistent results may be attributed to differences in product quality, supplementation dosage and duration, experimental period, and animal age. Under the conditions of this experiment, compared with the control group, the 250 mg/kg Clostridium butyricum group showed no significant difference in ADG but significantly lower ADFI, with F/G decreasing by 12.78%, suggesting that Clostridium butyricum improved nutrient digestion and absorption and growth performance. Compared with the 250 and 500 mg/kg groups, the 1,000 and 2,000 mg/kg groups showed no significant improvement in growth performance, possibly because excessive Clostridium butyricum competed with beneficial intestinal bacteria for nutrients and colonization sites, reducing available nutrients for the host and compromising intestinal microecological balance.

Weaned piglets experience stress from nutrition, psychology, and environment, manifesting as indigestion, diarrhea, growth retardation, and weakened disease resistance, which can be fatal in severe cases [19]. Clostridium butyricum can stimulate mucosal immunity [20], enhance immunity and disease resistance, antagonize pathogens, maintain and regulate intestinal microecological balance [21], and effectively alleviate piglet diarrhea. The present results confirmed these effects, showing that Clostridium butyricum significantly reduced diarrhea rate in weaned piglets, consistent with Xiao et al. [16]. Xiao et al. [22] found that Clostridium butyricum improved growth performance and reduced mortality in partridge chickens, and similar conclusions were drawn in this experiment.

3.2 Effects of Clostridium butyricum on Intestinal Structure of Weaned Piglets

Weaning stress causes intestinal damage, characterized by reduced villus height, deepened crypts, transformation of intestinal morphology from dense, finger-like villi to smooth, tongue-like surfaces, and decreased active absorption capacity [23]. Pang et al. [15] reported that 500 mg/kg Clostridium butyricum significantly reduced ileal crypt depth and increased villus height to crypt depth ratio in weaned piglets. The present results showed that Clostridium butyricum significantly increased jejunal villus height and villus height to crypt depth ratio, possibly because butyric acid produced by Clostridium butyricum metabolism serves as a primary nutrient for intestinal epithelial cell regeneration and repair, promoting intestinal epithelial regeneration [6]. As a normal intestinal flora, dietary Clostridium butyricum may also improve intestinal flora imbalance caused by weaning and alleviate pressure on intestinal structure repair from adverse environments.

3.3 Effects of Clostridium butyricum on Immune Function of Weaned Piglets

Chen et al. [24] reported that compound probiotics (containing Clostridium butyricum) significantly increased liver and spleen indexes in weaned piglets. Deng [25] found that Clostridium butyricum alone or glutamine alone had no significant effect on liver and spleen indexes, but their combination significantly increased these indexes compared with the control group. The present results showed that Clostridium butyricum had no significant effect on liver and spleen indexes, suggesting minimal or no impact on these parameters in weaned piglets.

Immunoglobulins are the primary antibodies mediating humoral immune responses. They bind to specific antigens to elicit immune reactions and protect the organism, and their increased content indicates enhanced immunity. Wang [26] found that Clostridium butyricum ZJU-1 tended to increase serum IgG, IgM, and C4 contents and significantly increased IgA and C3 contents in weaned piglets. The present results were similar, showing that Clostridium butyricum significantly increased serum IgA and IgG contents compared with the control group. These results suggest that dietary Clostridium butyricum may enhance immune function by stimulating IgA and IgG secretion.

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