Postprint: Clostridium butyricum Attenuates Lipopolysaccharide-Induced Intestinal Injury in Acutely Stressed Rats

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

Abstract

This experiment aimed to investigate the effects of Clostridium butyricum on growth performance in healthy rats and on intestinal structure, intestinal disaccharidase activity, and intestinal inflammation in rats with LPS-induced acute stress. Thirty-six male SD rats were selected and divided into control, LPS, and LPS+Clostridium butyricum groups based on body weight, with 4 rats per cage and 3 cages per group. The control and LPS groups were fed a basal diet, while the LPS+Clostridium butyricum group received the basal diet supplemented with 0.05% Clostridium butyricum. On day 40 of the experiment, rats in the LPS and LPS+Clostridium butyricum groups were weighed and injected intraperitoneally with LPS at a dose of 4 mg/kg BW (LPS concentration 1.5 mg/mL, injection volume approximately 1.0 mL), while control rats received an intraperitoneal injection of 1 mL normal saline; samples were collected 3 hours later after sacrifice. The results showed: 1) Clostridium butyricum had no significant effect on final body weight, average daily gain, average daily feed intake, or feed-to-gain ratio in healthy rats ($P<0.05$). 2) Compared with the control group, LPS injection significantly increased duodenal crypt depth ($P<0.05$), and significantly decreased duodenal villus-to-crypt ratio, jejunal mucosal thickness, and the number of intraepithelial lymphocytes in the jejunum and ileum ($P<0.05$). Compared with the LPS group, prophylactic feeding of Clostridium butyricum significantly decreased duodenal crypt depth ($P<0.05$), and significantly increased jejunal mucosal thickness and the number of intraepithelial lymphocytes ($P<0.05$). 3) Compared with the control group, LPS injection significantly decreased duodenal sucrase and jejunal maltase activities ($P<0.05$). Compared with the LPS group, prophylactic feeding of Clostridium butyricum inhibited the LPS-induced decrease in duodenal sucrase activity; the duodenal sucrase activity in the LPS+Clostridium butyricum group showed no significant difference compared with the control group ($P>0.05$). 4) Compared with the

control group, LPS injection significantly increased myeloperoxidase (MPO) activity and interleukin-6 (IL-6) and tumor necrosis factor- (TNF-) contents in the jejunum and ileum (P<0.05). Compared with the LPS group, prophylactic feeding of Clostridium butyricum significantly decreased MPO activity in the jejunum and ileum and IL-6 and TNF- contents in the jejunum (P<0.05); although IL-6 and TNF- contents in the ileum of the LPS+Clostridium butyricum group showed no significant decrease (P>0.05), they also showed no significant increase compared with the control group (P>0.05). In conclusion, Clostridium butyricum had no negative effects on growth in healthy rats, and prophylactic feeding of Clostridium butyricum to rats could inhibit the LPS-induced decrease in intestinal sucrase activity under acute LPS stress, alleviate damage to intestinal mucosal structure, enhance intestinal immune function, and reduce intestinal inflammation.

Full Text

Inhibitory Effect of Clostridium butyricum on Intestinal Damage Induced by Lipopolysaccharide in Acutely Stressed Rats

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Abstract

This experiment was conducted to investigate the effects of *Clostridium butyricum* on growth performance in healthy rats and on intestinal structure, intestinal disaccharidase activities, and intestinal inflammation in rats with lipopolysaccharide (LPS)-induced acute stress. Thirty-six male SD rats were randomly allocated to three groups: a control group, an LPS group, and an LPS+*Clostridium butyricum* group, with 4 rats per cage and 3 cages per group. The control and LPS groups were fed a basal diet, while the LPS+*Clostridium butyricum* group received the basal diet supplemented with 0.05% *Clostridium butyricum*. On day 40 of the experiment, rats in the LPS and LPS+*Clostridium butyricum* groups were intraperitoneally injected with LPS at a dose of 4 mg/kg body weight (LPS concentration: 1.5 mg/mL, injection volume: approximately 1 mL). Control rats received an intraperitoneal injection of 1 mL saline solution. All rats were euthanized for sample collection 3 hours post-injection.

The results showed that: (1) *Clostridium butyricum* had no significant effects on final body weight, average daily gain, average daily feed intake, or feed-to-gain ratio in healthy rats (P>0.05). (2) Compared with the control group, LPS injection significantly increased crypt depth in the duodenum (P<0.05) and

significantly decreased the villus height-to-crypt depth ratio in the duodenum, mucosal thickness in the jejunum, and intraepithelial lymphocyte (IEL) counts in the jejunum and ileum (P<0.05). Preventive administration of *Clostridium butyricum* significantly reduced duodenal crypt depth (P<0.05) and significantly increased jejunal mucosal thickness and IEL counts (P<0.05) compared with the LPS group. (3) LPS injection significantly decreased sucrase activity in the duodenum and maltase activity in the jejunum compared with the control group (P<0.05). Preventive administration of *Clostridium butyricum* inhibited the LPS-induced reduction in duodenal sucrase activity, with no significant difference observed between the LPS+*Clostridium butyricum* group and the control group (P>0.05). (4) LPS injection significantly increased myeloperoxidase (MPO) activity and interleukin-6 (IL-6) and tumor necrosis factor- (TNF-) contents in the jejunum and ileum compared with the control group (P<0.05). Preventive administration of *Clostridium butyricum* significantly decreased MPO activity in the jejunum and ileum and reduced IL-6 and TNF- contents in the jejunum (P<0.05). Although IL-6 and TNF- contents in the ileum of the LPS+*Clostridium butyricum* group showed no significant decrease compared with the LPS group (P>0.05), they also did not differ significantly from the control group (P>0.05).

In conclusion, *Clostridium butyricum* shows no negative effects on the growth performance of healthy rats. Preventive administration of *Clostridium butyricum* can inhibit LPS-induced reduction of intestinal sucrase activity, alleviate damage to intestinal mucosal structure, enhance intestinal immune function, and reduce intestinal inflammation in rats under acute stress.

Keywords: *Clostridium butyricum*; rat; growth performance; intestine; inflammation; lipopolysaccharide

Introduction

In livestock production, stress factors such as weaning often cause intestinal inflammation and damage to intestinal barrier function, resulting in diarrhea and digestive disorders. Probiotics are beneficial active microorganisms that can colonize the host and improve intestinal microecological balance. In animal production, probiotics are commonly used as growth promoters to help establish and improve intestinal microbial flora, increase feed efficiency, enhance immunity, and improve product quality, representing a promising alternative to antibiotics [1-2]. Various probiotic species exist with different efficacies. *Clostridium butyricum* is an anaerobic Gram-positive spore-forming bacterium that exists primarily as endospores, making it easy to store and highly resistant to adverse conditions. Research indicates that *C. butyricum* can produce various digestive enzymes, vitamins, butyric acid, and other nutrients, demonstrating excellent intestinal nutritional functions [3]. Additionally, its metabolic products—including butyric acid, acetic acid, and hydrogen—can reduce intestinal inflammation

and accelerate intestinal damage repair [4-5]. The bacterium shows promising applications for weaning-induced diarrhea, intestinal damage, and intestinal flora disorders, and was approved by the Ministry of Agriculture in July 2009 as a new generation of microecological feed additive.

Currently, *C. butyricum* is used preventively by adding it to diets, but research on its application effects in healthy animals during acute stress-induced damage remains limited. Whether preventive supplementation can help healthy hosts alleviate acute stress injury requires further investigation. Therefore, this study used rats as a model to evaluate the effects of preventive *C. butyricum* administration on growth performance in healthy rats and its protective effects against LPS-induced acute stress on intestinal structure, disaccharidase activities, and intestinal inflammation, aiming to provide a theoretical basis for using preventive *C. butyricum* supplementation to mitigate acute stress damage in animals.

Materials and Methods

1.1 Experimental Animals and Materials

The *Clostridium butyricum* preparation (4×10 CFU/g) was provided by the Institute of Plant Microbiology, Zhejiang Academy of Agricultural Sciences. Thirty-six male SD rats weighing approximately 120 g were purchased from the Zhejiang Academy of Medical Sciences. The basal diet was a maintenance rat chow also obtained from the Zhejiang Academy of Medical Sciences, with composition and nutrient levels shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients (g/kg)	
Corn starch	
Casein	
Soybean oil	
Sucrose	
Dextrin	
Cellulose	
Vitamin premix[1]	
Mineral premix[2]	
Cys	
Choline chloride	
Total	
Nutrient levels	
Gross energy (MJ/kg)	
Crude protein (%)	
Ether extract (%)	

Items	Content
Nitrogen-free extract (%)	
Crude fiber (%)	
Crude ash (%)	

[1]The vitamin premix provided the following per kg of diet: VA 4,000 IU, VD 1,000 IU, VE 75 IU, VK 0.9 mg, VB 5 mg, VB 6 mg, VB 30 mg, VB 15 mg, VB 6 mg, choline 1,000 mg, folic acid 2 mg, biotin 0.2 mg, VB 0.025 mg.

[2]The mineral premix provided the following per kg of diet: Ca 5,000 mg, P 3,000 mg, K 3,600 mg, Na 1,039 mg, Mg 513 mg, Fe 45 mg, Zn 38 mg, Mn 10 mg, Cu 6 mg, I 0.2 mg, Cr 1 mg, S 300 mg, Cl 1,631 mg.

1.2 Experimental Design and Management

SD rats were randomly divided into three groups according to body weight: control, LPS, and LPS+*Clostridium butyricum*, with 4 rats per cage and 3 cages per group. Room temperature was maintained at 25°C, and bedding was changed every 3 days to keep cages clean and dry. All rats had free access to water and feed. After a 3-day adaptation period, the experiment began. The control and LPS groups received the basal diet, while the LPS+*C. butyricum* group received the basal diet supplemented with 0.05% *C. butyricum*. Body weight and feed intake were recorded every 5 days. On day 40, all rats were weighed, and those in the LPS and LPS+*C. butyricum* groups received an intraperitoneal injection of LPS at 4 mg/kg body weight (1.5 mg/mL concentration, approximately 1 mL volume). The control group received 1 mL saline solution. All rats were euthanized 3 hours post-injection, and small intestinal samples were collected.

1.3 Sample Collection and Detection Indicators

Rats were euthanized by cervical dislocation. The abdominal cavity was opened, and intestinal tissues were isolated. Duodenum, jejunum, and ileum tissues were collected in duplicate. One portion of intestinal tissue was fixed overnight in 4% paraformaldehyde, then paraffin-embedded, sectioned serially, and stained with hematoxylin-eosin (HE) for microscopic examination (Olympus DP-71 imaging system). Image-Pro Plus 5.0 software was used to capture images and measure villus height, crypt depth, mucosal thickness, and intraepithelial lymphocyte (IEL) count, and to calculate the villus height-to-crypt depth ratio (V/C = villus height/crypt depth). IEL counting was performed as follows: 3-6 villi were observed per specimen, and lymphocytes per 100 columnar epithelial cells in each villus epithelial layer were counted and converted to percentage (%), with the average representing the IEL count for that specimen. The other portion of intestinal tissue was placed in centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Intestinal total protein content and activities of myeloperoxidase (MPO), sucrase, lactase, and maltase were determined using colorimetric methods. Interleukin-6 (IL-6) and tumor necrosis factor- (TNF-) contents were measured using enzyme-linked immunosorbent assay (ELISA) kits. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4 Data Processing

All experimental data are expressed as mean ± standard deviation. One-way ANOVA was performed using SPSS 17.0 software, with LSD multiple comparison tests used to detect significant differences among the three groups. $P<0.05$ was considered statistically significant.

Results

2.1 Effects of *Clostridium butyricum* on Growth Performance in Healthy Rats

As shown in Table 2 , body weight changes were consistent across all groups, and *C. butyricum* had no significant effects on average daily feed intake, average daily gain, or feed-to-gain ratio ($P>0.05$).

Table 2 Effects of *Clostridium butyricum* on growth performance of healthy rats

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Initial weight (g)	117.17±9.87	119.66±7.89	118.00±5.46
Final weight (g)	386.00±35.16	377.50±30.62	389.50±15.24
Average daily gain (g/d)	6.63±1.00	6.38±0.77	6.84±0.45
Average daily feed intake (g/d)	23.95±0.58	24.21±0.78	25.06±1.02
Feed/gain	3.67±0.60	3.77±0.38	3.68±0.16

In the same row, values with different lowercase letter superscripts indicate

significant differences (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of *Clostridium butyricum* on Intestinal Structure and Intraepithelial Lymphocyte Count in LPS-Treated Rats

As shown in Table 3 , compared with the control group, LPS injection significantly increased duodenal crypt depth (P<0.05) and significantly decreased the villus height-to-crypt depth ratio in the duodenum (P<0.05), though no significant damage to villus structure was observed in the jejunum or ileum (P>0.05). Preventive administration of *C. butyricum* significantly reduced duodenal crypt depth compared with the LPS group (P<0.05) but did not prevent the LPS-induced decrease in the duodenal villus height-to-crypt depth ratio, which remained significantly lower than the control group (P<0.05). LPS injection significantly decreased jejunal mucosal thickness and IEL counts in the jejunum and ileum compared with the control group (P<0.05). Preventive administration of *C. butyricum* effectively inhibited these LPS-induced effects on mucosal thickness and IEL counts, with the most pronounced effects observed in the jejunum. The LPS+*C. butyricum* group showed significantly increased jejunal mucosal thickness and IEL counts compared with the LPS group (P<0.05), reaching levels comparable to the control group (P>0.05).

Table 3 Effects of *Clostridium butyricum* on intestinal structure and IEL count of LPS-treated rats

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Duodenum			
Villus height (m)	698.20±172.91	579.52±88.99	660.07±43.66
Crypt depth (m)	329.05±92.64	358.47±23.21	454.29±58.82
Villus height/Crypt depth	2.21±0.19	1.69±0.12	1.56±0.28
Mucosal thickness (m)	1,202.08±190.80	1,053.42±213.04	1,080.46±63.39
IEL count (%)	13.75±2.22	10.38±3.04	10.60±1.95
Jejunum			

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Villus height (m)	502.19±180.36	400.44±86.70	474.49±80.68
Crypt depth (m)	232.46±51.26	312.47±71.34	234.83±12.10
Villus height/Crypt depth	2.18±0.49	1.32±0.23	2.06±0.39
Mucosal thick-ness (m)	883.72±119.28	682.31±77.93	715.49±80.24
IEL count (%)	22.00±2.16	23.75±2.22	16.25±1.71
Ileum			
Villus height (m)	390.19±48.39	312.23±26.75	280.85±44.06
Crypt depth (m)	312.47±71.34	233.82±37.05	233.82±37.05
Villus height/Crypt depth	1.27±0.09	2.39±0.27	1.53±0.03
Mucosal thick-ness (m)	691.25±74.28	661.86±99.33	661.86±99.33
IEL count (%)	16.75±2.75	18.63±3.47	21.00±1.73

2.3 Effects of *Clostridium butyricum* on Intestinal Sucrase, Lactase, and Maltase Activities in LPS-Treated Rats

As shown in Table 4 , LPS injection significantly decreased duodenal sucrase activity and jejunal maltase activity compared with the control group ($P<0.05$). Activities of other disaccharidases in different intestinal segments showed decreasing trends but without significant differences ($P>0.05$). Preventive administration of *C. butyricum* inhibited the LPS-induced reduction in duodenal sucrase activity, with no significant difference observed between the LPS+*C. butyricum* group and the control group ($P>0.05$).

Table 4 Effects of *Clostridium butyricum* on intestinal disaccharidase activities of LPS-treated rats (U/mg prot)

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Duodenum			
Sucrase	8.24±2.28	5.74±0.34	10.61±0.61
Lactase	9.36±3.27	6.22±1.65	9.85±0.69
Maltase	45.96±28.45	23.79±9.06	37.45±5.78
Jejunum			
Sucrase	7.33±1.00	6.71±0.81	8.29±0.87
Lactase	8.29±0.87	6.80±1.98	6.80±1.98
Maltase	28.03±6.50	54.19±26.47	23.74±5.58
Ileum			
Sucrase	6.04±1.50	5.33±0.13	5.41±2.84
Lactase	6.15±0.94	4.64±1.03	4.87±1.77
Maltase	15.63±3.07	14.87±0.41	15.27±5.47

2.4 Effects of *Clostridium butyricum* on Intestinal Inflammation in LPS-Treated Rats

As shown in Table 5 , LPS injection significantly increased MPO activity and IL-6 and TNF- contents in the jejunum and ileum compared with the control group (P<0.05). Preventive administration of *C. butyricum* effectively suppressed inflammation in the jejunum and ileum. Compared with the LPS group, the LPS+*C. butyricum* group showed significantly decreased MPO activity in both the jejunum and ileum, as well as significantly reduced IL-6 and TNF- contents in the jejunum (P<0.05). Although IL-6 and TNF- contents in the ileum of the LPS+*C. butyricum* group showed no significant decrease compared with the LPS group (P>0.05), they also did not differ significantly from the control group (P>0.05).

Table 5 Effects of *Clostridium butyricum* on intestinal MPO, IL-6 and TNF- contents of LPS-treated rats

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Duodenum			
MPO (U/g prot)	1.92±0.34	2.20±0.42	2.75±0.88
IL-6 (ng/g prot)	3.63±0.55	3.78±0.64	4.04±1.05
TNF- (ng/g prot)	15.67±2.29	18.34±2.73	18.48±4.20

Items	Control group	LPS group	LPS+*Clostridium butyricum* group
Jejunum			
MPO (U/g prot)	1.52±0.12	1.99±0.12	1.22±0.08
IL-6 (ng/g prot)	2.70±0.08	3.45±0.43	2.31±0.21
TNF- (ng/g prot)	12.93±0.14	16.91±0.67	11.35±1.20
Ileum			
MPO (U/g prot)	1.03±0.23	2.44±0.58	1.54±0.15
IL-6 (ng/g prot)	2.76±0.64	3.74±0.91	2.91±0.27
TNF- (ng/g prot)	10.21±1.12	18.18±5.34	13.41±0.56

Discussion

3.1 Effects of *Clostridium butyricum* on Growth Performance in Healthy Rats

Probiotics can regulate intestinal flora and produce beneficial substances in the host intestine, positively influencing body weight. In rats fed high-fat diets, *Lactobacillus* supplementation reduced body weight and inhibited obesity by modulating intestinal flora structure and increasing blood leptin levels [6]. In animal production, probiotics are often used as growth promoters to mitigate environmental or feed stress. Meng et al. [7] found that supplementation with *Bacillus subtilis* and *C. butyricum* effectively improved average daily gain and feed-to-gain ratio in growing-finishing pigs throughout the entire period, while also significantly increasing apparent digestibility of gross energy and nitrogen. Cao et al. [8] reported that adding *C. butyricum* at 2.5×10 or 5×10 CFU/kg to broiler diets significantly improved average daily gain and feed-to-gain ratio at 21 and 42 days of age, with effects comparable to antibiotic supplementation. However, probiotic effects on body weight are not always significant. Kuo et al. [9] found that probiotics had limited effects on body weight and feed intake in healthy mice during 3-4 week trials, consistent with our findings. This study found that *C. butyricum* supplementation had no significant effects on growth

parameters including average daily gain and feed-to-gain ratio in healthy rats. This may be related to animal health status and environmental conditions. The rats used in this experiment were in a rapid growth phase, and the cage environment was clean and hygienic, which may have masked the growth-promoting effects of probiotics.

3.2 Effects of *Clostridium butyricum* on Intestinal Structure and Intraepithelial Lymphocyte Count in LPS-Treated Rats

Villus height, crypt depth, villus height-to-crypt depth ratio, and mucosal thickness are important indicators reflecting intestinal development and nutrient absorption. Wang [10] demonstrated that *C. butyricum* ZJU-F1 could inhibit weaning stress-induced reductions in villus height and villus height-to-crypt depth ratio in the duodenum, jejunum, and ileum, with piglets fed *C. butyricum* showing more orderly and dense microvilli structures under light microscopy. Liu [11] obtained similar results, showing that adding 1,000 mg/kg *C. butyricum* to broiler diets significantly increased villus height and villus height-to-crypt depth ratio in the duodenum, jejunum, and ileum, while increasing jejunal mucosal thickness. Our study found that *C. butyricum* feeding effectively reduced duodenal crypt depth and increased jejunal mucosal thickness, consistent with previous research. This intestinal nutritional function of *C. butyricum* may be related to its metabolic products. During fermentation, *C. butyricum* produces glycosidases, cellulases, vitamins, and butyric acid, with butyric acid particularly providing energy for intestinal mucosal cells and accelerating their proliferation and maturation [3,12].

Intraepithelial lymphocytes distributed among intestinal mucosal epithelial cells constitute the first line of intestinal immune defense, directly participating in mucosal immune responses against pathogen invasion and accelerating epithelial cell regeneration through pseudopodia contact [13]. Increased IEL counts help enhance immune capacity [14]. This study found that after 40 days of dietary *C. butyricum* supplementation, the LPS+*C. butyricum* group showed significantly increased jejunal IEL counts compared with the LPS group, reaching control group levels. These results align with previous studies. Rieger et al. [15] found that feeding *Enterococcus faecium* to weaned piglets significantly increased IEL counts at apical and nuclear positions within the epithelium. Bai et al. [16] also reported that dietary supplementation with 0.1% or 0.2% probiotics significantly increased intestinal IEL counts, particularly T lymphocyte counts, in broilers at 21 and 42 days of age. These findings indicate that feeding *C. butyricum* and other probiotics can enhance intestinal mucosal immunity, particularly cellular immunity.

3.3 Effects of *Clostridium butyricum* on Intestinal Disaccharidase Activities in LPS-Treated Rats

Animals primarily obtain energy from carbohydrates, whose digestion and absorption depend mainly on small intestinal disaccharidases. Disaccharidase ac-

tivity can be used to measure the developmental status of intestinal mucosal epithelial cells [17]. Dou [18] reported that dietary supplementation with *Lactobacillus* at 6×10 CFU/g effectively alleviated LPS-induced reductions in lactase activity in the jejunum and ileum. In mice infected with *Giardia*, administration of *Lactobacillus* before or during infection significantly increased intestinal sucrase and lactase activities [19]. Our study found that *C. butyricum* feeding showed a trend toward increasing sucrase and lactase activities in rat intestines.

3.4 Effects of *Clostridium butyricum* on Intestinal Inflammation in LPS-Treated Rats

TNF- and IL-6 are important inflammatory mediators involved in immune responses. Their increased production can trigger a cascade release of other inflammatory factors, causing cellular damage [20-21]. MPO is a heme protease secreted by neutrophils and macrophages, with its activity reflecting the degree of intestinal inflammatory infiltration. This study found that preventive *C. butyricum* feeding effectively reduced MPO activity and IL-6 and TNF- contents in the jejunum and ileum, with IL-6 and TNF- levels in the ileum not significantly different from the control group. These results demonstrate that *C. butyricum* can effectively inhibit LPS-induced intestinal inflammation, consistent with previous studies. Zhang et al. [22] found that daily gavage with 2 mL *C. butyricum* (2.3×10^{11} CFU/L) for 21 days effectively reduced blood IL-23 and TNF- contents and inhibited oxazolone-induced colonic inflammation in a rat colitis model. Pang et al. [23] also reported that oral administration of *C. butyricum* for 8 days effectively reduced serum endotoxin and D-lactate levels and decreased ileal IL-10 mRNA expression in diarrheal rats. The anti-inflammatory effects of *C. butyricum* are primarily mediated through its metabolic products butyric acid and hydrogen. Research shows that butyric acid can promote epithelial cell proliferation, accelerate damage repair, and reduce inflammation [4], while hydrogen has recently been identified as an anti-inflammatory gas that can alleviate oxidative stress and treat various inflammatory diseases [5,24]. Additionally, *C. butyricum* can stimulate macrophage aggregation near inflammatory sites and activate IL-10 release through the Toll-like receptor 2 (TLR2)/myeloid differentiation factor 88 (MyD88) signaling pathway, thereby inhibiting inflammatory factor production [25].

Conclusion

1. Under the conditions of this experiment, dietary supplementation with 0.05% *Clostridium butyricum* had no significant effect on the growth performance of healthy rats.
2. Preventive supplementation of *Clostridium butyricum* in rat diets can inhibit LPS-induced reduction of intestinal sucrase activity, alleviate LPS-induced damage to intestinal villus height and crypt depth, improve in-

testinal immune function, and reduce intestinal inflammation.

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