# Effects of Differently Processed Soybean Meal and Lactobacillus acidophilus Culture on Growth Performance and Intestinal Morphology and Structure in Weaned Piglets: Postprint

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## Abstract

This study aimed to investigate the effects of differently processed soybean meals and Lactobacillus acidophilus culture on the growth performance and intestinal morphology of weaned piglets. A total of 240 21-day-old weaned piglets with similar genetic background, parity, and body weight were selected and randomly divided into 5 groups, with 4 replicates per group and 12 piglets per replicate. Piglets in the control group were fed a corn-soybean meal basal diet, those in the fermented soybean meal group were fed a diet in which 10% fermented soybean meal replaced part of the conventional soybean meal, those in the extruded soybean group were fed a diet in which extruded soybean replaced all conventional soybean meal, and those in the Lactobacillus acidophilus culture groups were fed diets supplemented with 3.0% and 5.0% Lactobacillus acidophilus culture, respectively. The digestible energy and crude protein levels were similar across all dietary groups. The experimental period lasted 21 days. The results showed that: 1) Compared with the control group, the feed-to-gain ratio of weaned piglets in the fermented soybean meal group was significantly decreased ($P<0.05$), and the average daily feed intake of weaned piglets in the 3% Lactobacillus acidophilus culture group was significantly increased ($P<0.05$). There was no significant difference in average daily feed intake between the 3% and 5% Lactobacillus acidophilus culture groups ($P>0.05$). 2) The villus height in the duodenum, jejunum, and ileum of weaned piglets in the 3% and 5% Lactobacillus acidophilus culture groups was significantly higher than that in the control and fermented soybean meal groups ($P<0.05$). The villus height/crypt depth (V/C) ratio in the duodenum, ileum, and jejunum of weaned piglets in the 3% and 5% Lactobacillus acidophilus culture groups was significantly higher than that in the control and fermented soybean meal groups ($P<0.05$). The V/C ratio in the duodenum of weaned piglets in the extruded soybean group was

significantly higher than that in the control group (P<0.05). 3) Transmission electron microscopy revealed that the microvilli in the jejunum of weaned piglets in the control group were unevenly distributed and varied in length, whereas those in the 3% and 5% Lactobacillus acidophilus culture groups were more densely packed and uniformly arranged. It can be concluded that dietary supplementation with fermented soybean meal or Lactobacillus acidophilus culture can improve the growth of weaned piglets, and dietary supplementation with Lactobacillus acidophilus culture can also improve intestinal morphology. Based on comprehensive evaluation of growth performance and intestinal morphology, the appropriate supplementation level of Lactobacillus acidophilus culture in weaned piglet diets is 3%.

## Full Text

# Effects of Different Processing Soybean Meal and Lactobacillus acidophilus Culture on Growth Performance and Intestinal Morphology of Weaned Piglets

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### Abstract

This study investigated the effects of different processing methods for soybean meal and *Lactobacillus acidophilus* culture on the growth performance and intestinal morphology of weaned piglets. A total of 240 21-day-old weaned piglets with similar genetic background, parity, and body weight were randomly allocated to five groups with four replicates per group and 12 piglets per replicate. The control group received a corn-soybean meal basal diet. In the fermented soybean meal group, 10% fermented soybean meal replaced a portion of conventional soybean meal. The expanded soybean group received a diet where all conventional soybean meal was replaced with expanded soybean. The *Lactobacillus acidophilus* culture groups were supplemented with either 3.0% or 5.0% *Lactobacillus acidophilus* culture. All diets were formulated to have similar levels of digestible energy and crude protein. The experimental period lasted 21 days. The results showed: (1) Compared with the control group, the feed-to-gain ratio (F/G) of piglets in the fermented soybean meal group was significantly reduced (P<0.05), while the average daily feed intake (ADFI) of piglets in the 3% *Lactobacillus acidophilus* culture group was significantly increased (P<0.05). No significant difference in ADFI was observed between the 3% and 5% *Lactobacillus acidophilus* culture groups (P>0.05). (2) The villus heights in the duodenum, jejunum, and ileum of piglets in both the 3% and 5% *Lactobacillus acidophilus* culture groups were significantly higher than those in the control and fermented

soybean meal groups (P<0.05). The villus height to crypt depth (V/C) ratios in the duodenum, jejunum, and ileum of piglets in the 3% and 5% *Lactobacillus acidophilus* culture groups were also significantly higher than those in the control and fermented soybean meal groups (P<0.05). The duodenal V/C ratio in the expanded soybean group was significantly higher than that in the control group (P<0.05). (3) Transmission electron microscopy revealed that the jejunal microvilli in the control group were unevenly distributed and varied in length, whereas those in the 3% and 5% *Lactobacillus acidophilus* culture groups were more densely packed and uniformly arranged. In conclusion, dietary supplementation with fermented soybean meal or *Lactobacillus acidophilus* culture can improve the growth of weaned piglets, and *Lactobacillus acidophilus* culture can additionally improve intestinal morphology. Based on comprehensive evaluation of growth performance and intestinal morphology, the optimal supplementation level of *Lactobacillus acidophilus* culture in weaned piglet diets is 3%.

**Keywords:** weaned piglets; *Lactobacillus acidophilus* culture; fermented soybean meal; intestinal morphology

## Introduction

Weaned piglets face multiple challenges during early weaning, including environmental, nutritional, and physiological changes. Due to their immature digestive systems, the large quantities of protein obtained from plant-based feed ingredients after weaning can cause digestive stress, leading to reduced appetite, indigestion, diarrhea, and even mortality [**?**]. This occurs because soybeans contain anti-nutritional factors such as trypsin inhibitors, soybean agglutinin, and urease, which can trigger allergic reactions in piglets, damage intestinal structure and function, impair digestive and absorptive capacity, and cause adverse physiological responses [**?**]. Consequently, appropriate processing measures are commonly employed in production to improve plant protein ingredients and enhance piglet health.

Research has shown that microbially fermented soybean meal possesses a unique fermented aroma that can stimulate feed intake. Furthermore, microorganisms secrete proteases that degrade protein-based anti-nutritional factors in soybean meal, thereby improving protein quality [**?**], resolving nutritional diarrhea issues [**?**], and positively affecting pig growth performance [**?**]. *Lactobacillus acidophilus* can regulate the microbial balance in the intestinal tract of livestock and poultry, promote the growth of beneficial bacteria, inhibit the proliferation of harmful bacteria, and consequently improve intestinal development and nutrient digestion and absorption. Abu Duorusuli·Aierken [**?**] found that *Lactobacillus acidophilus*-fermented soybean meal significantly increased the average daily gain (ADG) and reduced the feed-to-gain ratio (F/G) in yellow-feathered broilers. During the early post-weaning period, probiotics can improve piglet

growth performance and maintain intestinal health [**?**]. Given the characteristics of fermented soybean meal, expanded soybean, and *Lactobacillus acidophilus*, could incorporating these ingredients into diets improve soybean meal quality, promote piglet growth, and enhance intestinal health? And which supplementation ratio would be most effective? Therefore, this study was designed to investigate the effects of dietary supplementation with fermented soybean meal, expanded soybean, or different doses of *Lactobacillus acidophilus* culture on the growth performance and intestinal morphology of weaned piglets, providing a theoretical basis for the scientific application of fermented soybean meal and *Lactobacillus acidophilus* culture in weaned piglet diets.

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## Materials and Methods

### 1.1 Experimental Materials

Fermented soybean meal, expanded soybean, and *Lactobacillus acidophilus* culture were provided by Shanghai Yuanyao Biotechnology Co., Ltd. The crude protein contents of fermented soybean meal, expanded soybean, and *Lactobacillus acidophilus* culture were 50%, 35%, and 20%, respectively. The *Lactobacillus acidophilus* culture contained 11.9% moisture, 7.6% ash, 8.1% crude fiber, 6.2% lactic acid, and 8.4% acid-soluble protein.

### 1.2 Experimental Design

A total of 240 21-day-old weaned piglets with similar genetic background, parity, and body weight (half male and half female) were randomly divided into five groups with four replicates per group and 12 piglets per replicate. The experimental period lasted 21 days.

### 1.3 Experimental Diets

The control group received a corn-soybean meal basal diet. In the fermented soybean meal group, 10% fermented soybean meal replaced a portion of conventional soybean meal. The expanded soybean group received a diet where all conventional soybean meal was replaced with expanded soybean. The *Lactobacillus acidophilus* culture groups were supplemented with either 3.0% or 5.0% *Lactobacillus acidophilus* culture. All diets were formulated to have similar levels of digestible energy and crude protein. Diet formulations were based on the Chinese "Feeding Standard of Swine" (2004). The composition and nutrient levels of experimental diets are shown in Table 1 .

### 1.4 Animal Management

The experiment was conducted at a large-scale pig farm. Piglets were housed on nursery beds with room temperature maintained at 25–28°C and relative humidity at 55%–65%. All piglets had ad libitum access to feed and water

throughout the experimental period. Piglets were vaccinated according to routine procedures, and other daily management practices followed the farm' s standard protocols.

### 1.5 Sample Collection and Processing

On day 21 of the experiment, one piglet with body weight close to the replicate average and in good health was selected from each replicate, anesthetized with an intramuscular injection of 4% sodium pentobarbital, and euthanized by exsanguination via the jugular vein. The abdominal cavity was opened, and 5-cm segments from the middle of the duodenum, jejunum, and ileum were rapidly excised. The digesta was carefully rinsed with distilled water, and the intestinal wall was blotted dry with filter paper before fixation in 10% formalin solution for at least 24 hours. Additionally, approximately 2 cm of jejunal tissue was collected and fixed in pre-cooled 4% glutaraldehyde solution.

### 1.6 Measurements

**1.6.1 Growth Performance** Daily feed allocation was recorded throughout the experiment. Piglets were weighed on the morning of days 1 and 21 after overnight fasting. Average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated for each replicate.

**1.6.2 Intestinal Morphology** Middle segments of duodenum, jejunum, and ileum samples were processed through routine dehydration, paraffin embedding, sectioning, and hematoxylin-eosin (HE) staining. Crypt depth (CD) and villus height (VH) were measured using a double-blind method, with five fields observed per slide. The villus height to crypt depth (V/C) ratio was calculated.

**1.6.3 Jejunal Epithelial Ultrastructure** Approximately 2 cm of jejunal tissue was trimmed on ice into blocks of about 1 mm × 1 mm × 1 mm. Tissue blocks were transferred with forceps to vials containing pre-cooled 4% glutaraldehyde for primary fixation, washed three times with phosphate-buffered saline (PBS), post-fixed in 1% osmium tetroxide, washed three times with PBS, and dehydrated in a graded ethanol series (50%, 70%, 80%, 95%, and 100% twice) for 15 minutes each. Samples were then dehydrated twice in pure acetone (15 minutes each), infiltrated with Epon812:acetone (1:1) for 30 minutes, infiltrated with pure embedding medium for 1 hour, and polymerized at 37°C for 24 hours followed by 60°C for 48 hours. Ultrathin sections were cut using an ultramicrotome (BROMMA LKB-V), double-stained with uranyl acetate and lead citrate, and observed and photographed using a Hitachi H-600 transmission electron microscope.

### 1.7 Data Processing and Statistical Analysis

All experimental data were analyzed using one-way ANOVA in SPSS 16.0 software. Differences were considered significant at $P<0.05$. Data are expressed as "mean ± standard deviation."

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## Results

### 2.1 Effects on Growth Performance

The effects of different processing methods for soybean meal and *Lactobacillus acidophilus* culture on the growth performance of weaned piglets are presented in Table 2 . No significant differences in ADG were observed among the groups ($P>0.05$). The ADFI of piglets in the 3% *Lactobacillus acidophilus* culture group was significantly higher than that in the control, fermented soybean meal, and expanded soybean groups ($P<0.05$), but no significant difference was found between the 3% and 5% *Lactobacillus acidophilus* culture groups ($P>0.05$). The fermented soybean meal group exhibited the lowest F/G, which was significantly lower than that of the control and 5% *Lactobacillus acidophilus* culture groups ($P<0.05$), but not significantly different from the expanded soybean and 3% *Lactobacillus acidophilus* culture groups ($P>0.05$).

### 2.2 Effects on Intestinal Morphology

The effects of different processing methods for soybean meal and *Lactobacillus acidophilus* culture on intestinal morphology are shown in Table 3 . The villus heights in the duodenum, jejunum, and ileum of piglets in both the 3% and 5% *Lactobacillus acidophilus* culture groups were significantly higher than those in the control and fermented soybean meal groups ($P<0.05$). No significant differences in villus height were observed between the 3% and 5% *Lactobacillus acidophilus* culture groups ($P>0.05$). Compared with the control group, the fermented soybean meal and expanded soybean groups showed no significant differences in villus height in the duodenum, jejunum, or ileum ($P>0.05$).

The crypt depths in the duodenum and ileum of piglets in the expanded soybean and 3% *Lactobacillus acidophilus* culture groups were significantly lower than those in the control group ($P<0.05$), but not significantly different from the 5% *Lactobacillus acidophilus* culture and fermented soybean meal groups ($P>0.05$). No significant differences in jejunal crypt depth were observed among any groups ($P>0.05$).

The V/C ratios in the duodenum, jejunum, and ileum of piglets in the 3% and 5% *Lactobacillus acidophilus* culture groups were significantly higher than those in the control and fermented soybean meal groups ($P<0.05$). The duodenal V/C ratio in the expanded soybean group was significantly higher than that in the control group ($P<0.05$), but no significant differences were found in the jejunal

or ileal V/C ratios between the expanded soybean and control groups (P>0.05).

### 2.3 Effects on Jejunal Epithelial Ultrastructure

The effects of different processing methods for soybean meal and *Lactobacillus acidophilus* culture on jejunal epithelial ultrastructure are illustrated in Figure 1 [Figure 1: see original paper]. Compared with other groups, the control group exhibited jejunal epithelial microvilli that were unevenly distributed and varied in length, with some villi showing signs of shedding and damage. In contrast, piglets fed diets supplemented with fermented soybean meal, expanded soybean, or *Lactobacillus acidophilus* culture showed jejunal epithelial microvilli that were noticeably denser and more uniformly arranged, with consistent villus length. The 3% and 5% *Lactobacillus acidophilus* culture groups displayed the most dense, uniform, and slender jejunal epithelial microvilli among all treatment groups.

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## Discussion

### 3.1 Effects on Growth Performance

In commercial production, early weaning often subjects piglets to stress manifested as reduced feed intake, indigestion, and diarrhea [**?**]. The primary reason for this phenomenon is the adaptive transition from liquid sow milk to solid feed. Due to the immature digestive tract of weaned piglets, digestive enzymes secreted by the intestine are insufficient to fully digest nutrients and anti-nutritional factors from various feed ingredients. Soybean meal, as a major feed ingredient, is a good plant protein source, but practical experience indicates that weaned piglets still face considerable difficulty in digesting its large molecular proteins and destroying its anti-nutritional factors. Research has found that fermented soybean meal exhibits significantly improved digestibility, and when used in weaned piglet diets, it can substantially increase duodenal and jejunal total protease and trypsin activities, significantly improve ADG, and reduce F/G [**?**]. Under the conditions of this experiment, no significant differences in ADG were observed among groups. However, compared with the control group, the fermented soybean meal group showed a significantly reduced F/G, likely because fermentation decreased the levels of large molecular antigenic proteins and trypsin inhibitors [**?**], thereby improving nutrient absorption and utilization and consequently enhancing feed conversion efficiency. Additionally, dietary supplementation with *Lactobacillus acidophilus* culture increased feed intake, with the 3% *Lactobacillus acidophilus* culture group showing significantly higher ADFI than the control, fermented soybean meal, and expanded soybean groups. No significant difference in ADFI was observed between the two *Lactobacillus acidophilus* culture groups. These results are consistent with the findings of Zhang Yong et al. [**?**].

### 3.2 Effects on Intestinal Morphology

The small intestine is the primary site for nutrient digestion and absorption in piglets. Well-developed villus and microvillus structures can greatly increase the surface area of the intestinal wall, thereby enhancing nutrient absorption. However, after weaning, the morphological structure of the small intestinal villi is affected to varying degrees [**?**]. The transition from liquid milk to solid feed further exacerbates digestive stress in piglets. Weaning stress often causes small intestinal villus atrophy and crypt deepening, suppresses digestive enzyme activity, and results in diarrhea and growth stagnation [**?**]. Direct addition of probiotics to diets or fermentation of diets with probiotics before feeding can improve intestinal morphology, enhance growth performance, increase serum immunoglobulin M (IgM) content, and promote secretion of secretory immunoglobulin A (SIgA) in the intestinal mucosa [**?**]. Dietary supplementation with *Lactococcus lactis* not only promotes the development of intestinal mucosal villi but also improves piglet growth performance. Similar results were obtained in this study, where replacing part of the conventional soybean meal with fermented soybean meal promoted intestinal villus development in weaned piglets, and diets supplemented with 3% or 5% *Lactobacillus acidophilus* culture effectively improved intestinal morphology and reduced intestinal damage. In terms of intestinal morphology, *Lactobacillus acidophilus* culture supplementation produced the most significant improvements. Examination of jejunal villus ultrastructure revealed that piglets fed diets supplemented with fermented soybean meal, expanded soybean, or *Lactobacillus acidophilus* culture showed jejunal epithelial microvilli that were noticeably denser and more uniformly arranged than those in the control group. Supplementation with 3% and 5% *Lactobacillus acidophilus* culture resulted in the densest, most uniform, and most slender jejunal epithelial microvilli among all groups.

Based on these findings, we speculate that expansion treatment can eliminate or reduce anti-nutritional factors in soybeans to some extent, while microbial proteases can degrade anti-nutritional factors in soybean meal and improve protein quality. The removal of anti-nutritional factors reduces intestinal inflammation and prevents damage to the intestinal mucosa, while the integrity of small intestinal morphology promotes nutrient absorption and utilization and improves piglet growth performance. Second, the *Lactobacillus acidophilus* culture contains probiotics that produce large amounts of lactic acid after fermentation, which can promote the pre-digestion of food in the stomach. When the gastrointestinal tract is in an acidic state, the growth of most pathogenic bacteria in the intestine is inhibited, while the development of intestinal villi in piglets is promoted. This is the main reason why supplementation with *Lactobacillus acidophilus* culture produced superior results.

## Conclusion

1. Dietary supplementation with fermented soybean meal can significantly reduce the F/G of weaned piglets, while dietary supplementation with 3% *Lactobacillus acidophilus* culture can significantly increase the ADFI of weaned piglets.

2. Dietary supplementation with *Lactobacillus acidophilus* culture can improve intestinal morphology. Based on comprehensive evaluation of growth performance and intestinal morphology, the optimal supplementation level of *Lactobacillus acidophilus* culture in weaned piglet diets is 3%.

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