

## Effects of *Saccharomyces cerevisiae* and *Bacillus* on Apparent Nutrient Digestibility, Intestinal Morphology, and Intestinal Immunity in Finishing Pigs (Postprint)

**Authors:** Qin Hong, Cai Chuanjiang, Zhao Yan, Che Xiangrong, Hang Gao, Guo Liang

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### Abstract

This experiment aimed to investigate the effects of *Saccharomyces cerevisiae* and *Bacillus* on nutrient apparent digestibility, intestinal morphology, and intestinal immunity in finishing pigs. Seventy-two finishing pigs with a body weight of  $(62.50 \pm 0.83)$  kg, Duroc  $\times$  Landrace  $\times$  Yorkshire, were randomly allocated into three groups with four replicates per group and six pigs per replicate (half barrows and half gilts). The control group was fed a basal diet; the *Saccharomyces cerevisiae* group was fed the basal diet supplemented with 0.5 g/kg active dry yeast preparation (yeast viable count  $1.0 \times 10^{10}$  CFU/g); the *Bacillus* group was fed the basal diet supplemented with 0.1 g/kg *Bacillus* preparation (Baci CFU/g). The experimental period lasted 56 days. The results showed that: 1) Compared with the control group, the *Bacillus* group exhibited significantly increased apparent digestibility of calcium and phosphorus ( $P < 0.05$ ), while the *Saccharomyces cerevisiae* group showed significantly increased apparent digestibility of calcium ( $P < 0.05$ ); 2) Compared with the control group, the *Saccharomyces cerevisiae* group demonstrated significantly decreased jejunal crypt depth ( $P < 0.01$ ) and significantly increased jejunal villus height/crypt depth ratio ( $P < 0.01$ ); 3) Compared with the control group, the *Bacillus* group had significantly upregulated secretory immunoglobulin A content in both jejunum and ileum ( $P < 0.01$ ). In conclusion, dietary supplementation with *Saccharomyces cerevisiae* and *Bacillus* both improved nutrient digestion and absorption in finishing pigs, with *Saccharomyces cerevisiae* improving intestinal morphological structure and *Bacillus* increasing SIgA content and enhancing intestinal immune level.

## Full Text

### Effects of *Saccharomyces cerevisiae* and *Bacillus* on Apparent Nutrient Digestibility, Intestinal Morphological Structure, and Intestinal Immunity of Finishing Pigs

\*\*QIN Hong<sup>1</sup>, CAI Chuanjiang<sup>2</sup>, ZHAO Yan<sup>1</sup>, CHE Xiangrong<sup>1\*</sup>, GAO Hang<sup>1</sup>, GUO Liang<sup>3\*\*</sup>

(1. College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Shanxi Collaborative Innovation Center for High-Productive and Safe Livestock, Taigu 030801, China; 2. Northwest A&F University, Yangling 712100, China; 3. Tianjin Agricultural University, Tianjin 300384, China)

**Abstract:** This experiment was conducted to investigate the effects of *Saccharomyces cerevisiae* and *Bacillus* on apparent nutrient digestibility, intestinal morphological structure, and intestinal immunity in finishing pigs. Seventy-two crossbred (Duroc × Landrace × Yorkshire) finishing pigs with an initial body weight of  $(62.50 \pm 0.83)$  kg were randomly allocated to three groups with four replicates per group and six pigs per replicate. Pigs in the *Saccharomyces cerevisiae* group were fed the basal diet supplemented with 0.5 g/kg active dry yeast preparation ( $1.0 \times 10^9$  CFU/g viable yeast count), and those in the *Bacillus* group were fed the basal diet supplemented with 0.1 g/kg *Bacillus* preparation ( $1.0 \times 10^9$  CFU/g viable *Bacillus* count). The experimental period lasted 56 days. The results showed that: 1) Compared with the control group, the apparent digestibility of calcium and phosphorus in finishing pigs was significantly increased in the *Bacillus* group ( $P < 0.05$ ), while the apparent digestibility of calcium was significantly increased in the *Saccharomyces cerevisiae* group ( $P < 0.05$ ). 2) Compared with the control group, the crypt depth in the jejunum of finishing pigs was extremely significantly decreased ( $P < 0.01$ ) and the villus height/crypt depth ratio in the jejunum was extremely significantly increased in the *Saccharomyces cerevisiae* group ( $P < 0.01$ ). 3) Compared with the control group, the secretory immunoglobulin A (SIgA) content in both the jejunum and ileum of finishing pigs was extremely significantly upregulated in the *Bacillus* group ( $P < 0.01$ ). In conclusion, dietary supplementation with *Saccharomyces cerevisiae* and *Bacillus* can improve nutrient digestion and absorption in finishing pigs, with *Saccharomyces cerevisiae* showing beneficial effects on intestinal morphological structure, while *Bacillus* can increase SIgA content in the intestine and enhance intestinal immune function.

**Keywords:** yeast; *Bacillus*; finishing pig; apparent digestibility; intestinal morphological structure; intestinal cytokines

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Since their discovery, antibiotics have been used as drugs to maintain human and animal health [1]. Over the past 50 years, antibiotics have demonstrated clear effects in disease prevention and growth promotion in practical production, bringing enormous economic benefits to the livestock industry. However,

in the last two decades, with the development of science and technology and improvements in living standards, people have gradually recognized that the abuse of antibiotics can cause negative issues such as residues in livestock products, drug resistance, and environmental pollution [2-3]. Therefore, seeking green, efficient, pollution-free, and residue-free feed additives to replace antibiotics and chemically synthesized drugs has become an inevitable trend and an important research focus in feed science both domestically and internationally. Microecological preparations are considered one of the effective alternatives to antibiotics because they can improve animal immunity and production performance [4], with yeast and *Bacillus* being two commonly used microecological preparations [5].

Yeast is a general term for a class of single-celled eukaryotic microorganisms that can ferment sugars, representing a complex group. Studies have reported that adding active yeast to sow diets can improve piglet growth performance, immune status, weaning weight, and litter weight [6-7]. Yeast culture can exert beneficial effects on the intestinal health and immune system of growing pigs [8]. Research has found that *Saccharomyces cerevisiae*, as a beneficial bacterium, can effectively compete with and inhibit pathogen proliferation during its growth, regulate animal intestinal microecosystems, promote intestinal mucosal development, and improve nutrient digestion and absorption [9]. During fermentation production, yeast culture can produce components beneficial to animal intestinal microecological balance and immune function, including cell wall polysaccharides, active beneficial bacteria, secondary metabolites, minerals, vitamins, and unknown growth-promoting factors, making it a valuable research subject [10-11]. *Bacillus* is a class of aerobic or facultative anaerobic Gram-positive bacteria and a type of probiotic that can directly metabolize and produce various nutrients while secreting enzymes such as amylase and pectinase [12]. Under adverse environmental stress, *Bacillus* can exist in spore form, demonstrating acid and heat resistance [13]. It can improve dietary nutrient digestibility [14], enhance animal immunity [15], promote animal growth [16], antagonize pathogenic microorganisms [17], and reduce ammonia emission from excreta [18]. It has been widely applied in various livestock, poultry, and aquatic animal feeds and represents a probiotic with broad application prospects.

However, in the field of microecological preparation research, many scholars have focused on the mechanisms and comparative effects of strains but have not conducted extensive research on strain selection and performance comparison. Moreover, both yeast and *Bacillus* have been more frequently applied during the early weaning piglet stage [19-21], with limited research reports on the finishing pig stage. Therefore, this experiment used finishing pigs as the research object to investigate and compare the effects of these two microecological preparations on growth performance, nutrient apparent digestibility, intestinal morphological structure, and intestinal immunity through dietary supplementation.

### 1.1 Experimental Design

Seventy-two crossbred (Duroc × Landrace × Yorkshire) finishing pigs with a body weight of  $(62.50 \pm 0.83)$  kg were selected and randomly divided into three groups according to the principle of *Saccharomyces cerevisiae* group was fed the basal diets supplemented with 0.5 g/kg active dry yeast preparation ( $1.0 \times 10^9$  CFU/g viable yeast count), and the *Bacillus* group was fed the basal diet supplemented with 0.1 g/kg *Bacillus* preparation ( $1.0 \times 10^9$  CFU/g viable *Bacillus* count). The experimental period lasted 56 days. The basal diet was in powder form and was formulated according to the NRC (2012) nutrient requirements for pigs. Its composition and nutrient levels are shown in Table 1. During the experiment, pigs were fed four times daily with ad libitum access to feed and water. On day 56 of the experiment, feeding was withheld for 12 hours starting at 20:00, and one barrow per replicate with body weight close to the average was selected for slaughter.

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

| Item                                  | Content |
|---------------------------------------|---------|
| <b>Ingredients</b>                    |         |
| Corn                                  |         |
| Rice bran                             |         |
| Soybean meal                          |         |
| Cottonseed meal                       |         |
| Distillers dried grains with solubles |         |
| Limestone                             |         |
| CaHPO <sub>4</sub>                    |         |
| NaCl                                  |         |
| L-Lys · HCl                           |         |
| Choline chloride                      |         |
| Premix <sup>1</sup>                   |         |
| <b>Total</b>                          |         |
| <b>Nutrient levels<sup>2</sup></b>    |         |
| DE/(MJ/kg)                            |         |
| NE/(MJ/kg)                            |         |
| CP                                    |         |
| Ca                                    |         |
| TP                                    |         |
| Nonphytate P                          |         |
| Lys                                   |         |
| Met+Cys                               |         |

<sup>1</sup>The premix provided the following per kg of the diet: VA 8,800 IU, VB<sub>1</sub> 4 mg, VB<sub>2</sub> 8 mg, VB<sub>6</sub> 30 mg, VB<sub>12</sub> 0.020 mg, VD<sub>3</sub> 1,800 IU, VE 20 IU, VK<sub>3</sub> 4 mg, biotin 0.08 mg, calcium pantothenate 14 mg, nicotinic acid 28 mg, Cu (as copper sulfate) 20 mg, Fe (as ferrous sulfate) 60 mg, Mn (as manganese

sulfate) 40 mg, Zn (as zinc sulfate) 60 mg, I (as potassium iodide) 0.50 mg, Se (as sodium selenite) 0.30 mg.

<sup>2</sup>CP, Ca, and total P were measured values, while the others were calculated values.

## 1.2 Microbial Sources

The yeast was provided by Beijing Bangshifu Biotechnology Co., Ltd., with the strain being *Saccharomyces cerevisiae*, viable count  $2.0 \times 10^{10}$  CFU/g, and appropriate addition level of  $1.0 \times 10^{10}$  CFU/g.

The *Bacillus* was provided by Shandong Baolaili Bioengineering Co., Ltd., with strains being *Bacillus subtilis* and *Bacillus licheniformis*, viable count  $1.0 \times 10^{10}$  CFU/g, and appropriate addition level of  $1.0 \times 10^9$  CFU/g.

### 1.3.1 Growth Performance

At 07:00 on the first and last day of the experiment, pigs in each pen were weighed individually after fasting, and feed consumption was recorded. Average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated on a replicate basis.

### 1.3.2 Nutrient Apparent Digestibility

A digestion trial was conducted on day 35 of the experiment. Fecal samples were collected continuously for 4 days from each replicate, three times daily, with approximately 100 g collected each time from as many pigs as possible. Ten percent tartaric acid solution was added to prevent ammonia volatilization. Diet samples were also collected, dried at 65°C for 12 hours, equilibrated for 24 hours, and then ground. The crude protein, calcium, and phosphorus contents in diets and feces were determined according to the method in reference [22]. Nutrient apparent digestibility was calculated using the following formula:

$$\text{Nutrient apparent digestibility (\%)} = 100 \times [1 - (A/B) \times (C/D)]$$

where: A = nutrient content in feces; B = nutrient content in diet; C = acid-insoluble ash content in diet; D = acid-insoluble ash content in feces.

### 1.3.3 Intestinal Morphological Structure and Immune Cell Counting

After slaughter, 0.5 cm segments of the duodenum, jejunum, and ileum were aseptically collected, rinsed with physiological saline, and fixed in 4% paraformaldehyde. After paraffin embedding, 5 μm sections were cut and stained with hematoxylin-eosin (HE). Two non-consecutive sections were observed for each sample, with six typical fields selected per section. Villus height and crypt depth were measured using an optical microscope (RM2235 LEICA). Villus height was measured from the tip to the base, and crypt depth

was measured from the base of adjacent villi to the muscularis mucosae. The villus height/crypt depth ratio was calculated. For each pig, villi from six different intestinal segments were measured, and goblet cells were counted from the base in every 100 columnar epithelial cells [23]. Modified toluidine blue staining was used to observe mast cells in the intestinal mucosal lamina propria. Ten random fields were selected at 400× magnification, and mast cells in each field were counted [24].

### 1.3.4 Intestinal Cytokine Content

Sample pretreatment: Appropriate amounts of intestinal tissue were weighed, ground into powder in liquid nitrogen-precooled mortars, and then mixed with 9 volumes of cold physiological saline at a mass (g) to volume (mL) ratio of 1:9. The mixture was centrifuged at 4,000 r/min for 10 min, and the supernatant was collected in 1.5 mL EP tubes. The contents of secretory immunoglobulin A (SIgA), intestinal trefoil factor (ITF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-8 (IL-8) in intestinal tissue supernatants were determined using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kits purchased from Shanghai Xitang Biotechnology Co., Ltd.

### 1.4 Statistical Analysis

SPSS 19.0 software was used for one-way ANOVA, with Duncan's multiple comparison test applied. Results are expressed as means  $\pm$  standard error.  $P < 0.05$  was considered significant,  $P < 0.01$  extremely significant, and  $0.05 < P < 0.10$  indicated a significant trend.

## 2.1 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Growth Performance of Finishing Pigs

As shown in Table 2, there were no significant differences in ADFI, ADG, or F/G among groups ( $P > 0.05$ ).

**Table 2** Effects of *Saccharomyces cerevisiae* and *Bacillus* on growth performance of finishing pigs (n=12)

| Items               | Control group    | <i>Saccharomyces cerevisiae</i> group | <i>Bacillus</i> group | P-value |
|---------------------|------------------|---------------------------------------|-----------------------|---------|
| Initial weight/kg   | 62.41 $\pm$ 0.69 | 62.20 $\pm$ 1.26                      | 62.90 $\pm$ 2.48      |         |
| Final weight/kg     | 111.1 $\pm$ 1.99 | 112.6 $\pm$ 3.82                      | 112.7 $\pm$ 3.59      |         |
| ADFI/(kg weight/kg) |                  |                                       |                       |         |

In the same row, values with no letter or the same small letter superscripts mean no significant difference ( $P > 0.05$ ), while with different small letter superscripts mean significant difference ( $P < 0.05$ ), and with different capital letter superscripts mean extremely significant difference ( $P < 0.01$ ). The same as below.

## 2.2 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Nutrient Apparent Digestibility of Finishing Pigs

As shown in Table 3, compared with the control group, the apparent digestibility of calcium was significantly increased in the *Saccharomyces cerevisiae* group ( $P < 0.05$ ), while the apparent digestibility of both calcium and phosphorus was significantly increased in the *Bacillus* group ( $P < 0.05$ ). There were no significant differences in crude protein apparent digestibility among groups ( $P > 0.05$ ).

**Table 3** Effects of *Saccharomyces cerevisiae* and *Bacillus* on apparent nutrient digestibility of finishing pigs (n=12) %

| Items | Control group    | <i>Saccharomyces cerevisiae</i> group | <i>Bacillus</i> group | P-value  |
|-------|------------------|---------------------------------------|-----------------------|--|
| CP    | 76.48 $\pm$ 0.84 | 78.88 $\pm$ 0.85                      | 80.34 $\pm$ 1.56      | Ca 49.95 $\pm$ 0.36 <sup>b</sup>  53.63 $\pm$ 0.47 <sup>a</sup>  52.93 $\pm$ 1.12 <sup>a</sup>    P 41.06 $\pm$ 0.87 <sup>b</sup>  42.65 |

## 2.3 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Intestinal Morphological Structure of Finishing Pigs

Figure 1 [Figure 1: see original paper] shows the effects of *Saccharomyces cerevisiae* and *Bacillus* on intestinal morphological structure, while Figures 2 [Figure 2: see original paper], 3 [Figure 3: see original paper], and 4 [Figure 4: see original paper] show histological sections of the duodenum, jejunum, and ileum, respectively. As shown in Figure 1-a, there were no significant differences in villus height in the duodenum, jejunum, or ileum among groups ( $P > 0.05$ ). Figure 1-b indicates that compared with the control group, jejunal crypt depth was extremely significantly decreased in the *Saccharomyces cerevisiae* group ( $P < 0.01$ ). Figure 1-c shows that compared with the control group, the jejunal villus height/crypt depth ratio was extremely significantly increased in the *Saccharomyces cerevisiae* group ( $P < 0.01$ ).

**Figure 1** Effects of *Saccharomyces cerevisiae* and *Bacillus* on intestinal morphological structure of finishing pigs. Value columns with different small letters mean significant difference ( $P < 0.05$ ), with different capital letters mean extremely significant difference ( $P < 0.01$ ). The same as below.

**Figure 2** Slice atlases of duodenum (40 $\times$ )

**Figure 3** Slice atlases of jejunum (40 $\times$ )

**Figure 4** Slice atlases of ileum (40 $\times$ )

## 2.4 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Intestinal Cytokine Content of Finishing Pigs

As shown in Table 4, the SIgA content in the jejunum and ileum of pigs in the *Bacillus* group was extremely significantly higher than in the control and

*Saccharomyces cerevisiae* groups ( $P < 0.01$ ). Compared with the control group, IL-8 content in the jejunum showed a decreasing trend in the *Bacillus* group ( $0.05 < P < 0.10$ ), while IL-8 content in the ileum showed a decreasing trend in the *Saccharomyces cerevisiae* group ( $0.05 < P < 0.10$ ).

**Table 4** Effect of *Saccharomyces cerevisiae* and *Bacillus* on intestinal cytokine content of finishing pigs (n=12)

| Items                    | Control group                 | <i>Saccharomyces cerevisiae</i> group | <i>Bacillus</i> group         | P-value |
|--------------------------|-------------------------------|---------------------------------------|-------------------------------|---------|
| <b>Jejunum</b>           |                               |                                       |                               |         |
| SIgA/( $\mu\text{g/g}$ ) | 3.61 <sup>Bb</sup>            | 3.69 <sup>Bb</sup>                    | 6.38 <sup>Aa</sup>            |         |
| ITF/(ng/g)               | 2.23 $\pm$ 0.28               | 2.96 $\pm$ 0.12                       | 3.37 $\pm$ 0.62               |         |
| TNF- $\alpha$ /(ng/g)    | 0.046 $\pm$ 0.003             | 0.050 $\pm$ 0.004                     | 0.035 $\pm$ 0.004             | *       |
| <b>Ileum</b>             |                               |                                       |                               |         |
| SIgA/( $\mu\text{g/g}$ ) | 4.17 $\pm$ 0.21 <sup>Bb</sup> | 3.42 $\pm$ 0.44 <sup>Bb</sup>         | 6.12 $\pm$ 0.18 <sup>Aa</sup> |         |
| ITF/(ng/g)               | 2.81 $\pm$ 0.12               | 2.15 $\pm$ 0.29                       | 2.55 $\pm$ 0.3                |         |
| TNF- $\alpha$ /(ng/g)    | 0.039 $\pm$ 0.004             | 0.032 $\pm$ 0.006                     | 0.051 $\pm$ 0.004             |         |

## 2.5 Effects of *Saccharomyces cerevisiae* and *Bacillus* on the Number of Intestinal Immune Cells in Finishing Pigs

As shown in Table 5, there were no significant differences in the number of mast cells and goblet cells in the jejunum and ileum among groups ( $P > 0.05$ ).

**Table 5** Effects of *Saccharomyces cerevisiae* and *Bacillus* on the number of immune cells in intestine of finishing pigs (n=12)

| Items               | Control group   | <i>Saccharomyces cerevisiae</i> group | <i>Bacillus</i> group | P-value |
|---------------------|-----------------|---------------------------------------|-----------------------|---------|
| <b>Mast cells</b>   |                 |                                       |                       |         |
| Jejunum             | 8.17 $\pm$ 1.19 | 9.33 $\pm$ 3.20                       | 9.17 $\pm$ 2.04       |         |
| Ileum               | 9.83 $\pm$ 1.45 | 8.50 $\pm$ 1.65                       | 9.50 $\pm$ 0.96       | *       |
| <b>Goblet cells</b> |                 |                                       |                       |         |
| Jejunum             | 4.80 $\pm$ 0.42 | 5.80 $\pm$ 0.68                       | 6.50 $\pm$ 0.81       |         |
| Ileum               | 4.90 $\pm$ 0.59 | 4.60 $\pm$ 0.37                       | 5.90 $\pm$ 0.91       |         |

## 3.1 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Growth Performance of Finishing Pigs

Probiotics have been proven to play a positive role in improving feed conversion efficiency, promoting animal growth, and maintaining animal health in piglet production [19-21]. Yeast itself contains high levels of protein, amino acids, lysine, and vitamins, and its inherent characteristics can improve the ecological environment of the gastrointestinal tract in animals [25]. Many studies have confirmed that active yeast can improve the growth performance of finishing pigs. Cai et al. [26] found that adding 300 g/t of active yeast to diets of finishing

pigs weighing 90-100 kg significantly increased average daily gain and decreased feed-to-gain ratio. However, Huang et al. [27] reported that adding yeast culture to basal diets had no significant effect on average daily gain or average daily feed intake in finishing pigs during early or late fattening stages. After entering the gastrointestinal tract, *Bacillus* can metabolize and produce large amounts of digestive enzymes and nutrients for host utilization, thereby improving growth performance [12]. Studies by Jiang et al. [28] and Huo et al. [29] showed that adding *Bacillus* to finishing pig diets tended to improve growth performance, though the differences were not significant.

The present study showed that dietary supplementation with *Saccharomyces cerevisiae* and *Bacillus* had no significant effects on average daily gain, average daily feed intake, or feed-to-gain ratio in finishing pigs. This may be because the growth-promoting effects of probiotics are related to their ability to regulate intestinal microflora structure [30]. The intestinal development of finishing pigs is complete, and under fixed dietary conditions, the intestinal microflora has reached a relatively stable state [31]; therefore, exogenous microflora did not exert growth-promoting effects.

### 3.2 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Nutrient Apparent Digestibility of Finishing Pigs

Digestibility measurements can reflect both diet digestibility and animal digestive capacity [32]. After entering the intestine, *Bacillus* secretes highly active lipase, protease, cellulase, and amylase, which can promote nutrient digestion and absorption [12]. Studies by Jiang et al. [28] and Hua et al. [19] found that *Bacillus* preparations could improve the digestion and absorption of dietary crude protein, crude fat, crude fiber, and starch in pigs. Due to its rich content of protein, cellulose, minerals, B vitamins, and organic acids [33], dietary yeast supplementation can promote nutrient digestion and absorption in pigs [34-35]. However, Chen [20] reported that live yeast supplementation had no significant effect on apparent diet digestibility in weaned piglets. The present results showed that probiotics tended to improve the digestion and absorption of crude protein, calcium, and phosphorus in finishing pigs, with *Bacillus* supplementation significantly improving the apparent digestibility of calcium and phosphorus and showing a trend toward improved crude protein apparent digestibility, while *Saccharomyces cerevisiae* significantly improved calcium apparent digestibility but had no significant effect on crude protein or phosphorus apparent digestibility. The reason may be that *Bacillus* can produce large amounts of extracellular enzymes such as protease, amylase, and cellulase after entering the intestine, increasing enzyme activity in the intestine of finishing pigs and thereby promoting nutrient absorption [12]. Additionally, *Bacillus subtilis* can produce organic acids such as lactic acid after entering the intestine, and this acidic environment is conducive to releasing bound or chelated mineral elements in free form, thereby improving the utilization of calcium and phosphorus [36].

### 3.3 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Intestinal Morphological Structure of Finishing Pigs

The small intestine plays an important role in nutrient digestion, absorption, and transport [37]. Normal intestinal epithelial structure also plays a crucial role in intestinal barrier function and mucosal immune responses [38-39]. Intestinal villus height, crypt depth, and the ratio of villus height to crypt depth are important indicators for measuring intestinal digestion and absorption [40]. Small intestinal villi are the main sites for nutrient absorption; longer villi indicate higher nutrient absorption capacity [41]. Crypt depth reflects cell generation rate, and shallower crypts indicate increased cell maturation rate and enhanced secretory function. The villus height/crypt depth ratio comprehensively reflects small intestinal functional status; a higher value indicates larger intestinal membrane area and stronger digestion and absorption capacity [42].

Baum et al. [43] and Hu et al. [21] found that feeding yeast to weaned piglets significantly increased intestinal villus height. However, Bontempo et al. [44] observed obvious changes in villus height and crypt depth in piglets fed *Saccharomyces cerevisiae*, with a relatively low villus length/crypt depth ratio. Overall, reports on the effects of yeast on pig intestinal morphology have been inconsistent, with limited research on finishing pigs, and no consistent conclusions regarding the exact mechanism by which yeast improves small intestinal mucosal structure. The present study showed that adding  $1.0 \times 10^{10}$  CFU/g yeast decreased crypt depth in the jejunum and ileum and increased the villus height/crypt depth ratio in the jejunum and ileum of finishing pigs. This may be related to the  $\beta$ -glucan and mannan content in the yeast cell wall.  $\beta$ -glucan and mannan can reduce the binding of antigens to gastrointestinal mucosa by adsorbing, phagocytosing, destroying, and absorbing invading bacteria, thereby protecting the gastrointestinal mucosa from damage [45]. Chen [46] and Xin et al. [47] found that adding different doses of *Bacillus* to weaned piglet diets promoted small intestinal development. In contrast, the present study showed that adding  $1.0 \times 10^9$  CFU/g *Bacillus* had no significant effect on intestinal morphological structure in finishing pigs, which contradicts the above findings. This may be because the *Bacillus* added in this study was exogenous and the intestinal microflora of finishing pigs was stable, so the stimulatory effect of exogenous *Bacillus* on the intestine was less than that of endogenous bacteria [48]. The specific effects require further investigation.

### 3.4 Effects of *Saccharomyces cerevisiae* and *Bacillus* on Intestinal Cytokine Content and Immune Cell Numbers in Finishing Pigs

The intestinal mucosa is an important barrier against microorganisms and foreign substances in animals. The normal intestinal mucosal barrier is composed of mechanical, chemical, immune, and biological barriers. Cytokines are a class of protein substances with immunomodulatory functions produced by lympho-

cytes, mononuclear macrophages, and related immune cells, and are important information molecules in the immune system [49]. ITF is a small molecular protein secreted by goblet cells onto the intestinal mucosal surface that can promote intestinal mucosal cell proliferation and intestinal repair [50]. Mast cells are important immune-active cells that mainly participate in acquired immune responses by secreting cytokines [51]. The role of mast cells in defending against bacterial infections is mainly related to TNF- $\alpha$ ; in the early stages of bacterial invasion, TNF- $\alpha$  released by mast cells plays an important role in initiating defensive immune responses [52]. SIgA is an immunoglobulin A antibody found in external secretions and is the main antibody of mucosal immunity. It plays an important role in intestinal mucosal defense by preventing pathogen adhesion and clearing antigens without activating systemic immunity [53-54]. IL-8 is secreted by macrophages and epithelial cells, can chemoattract and activate neutrophils, cause mucosal edema, leukocyte infiltration, vascular damage, and increased permeability, leading to immune-inflammatory injury [55].

This experiment measured intestinal cytokine contents (ITF, SIgA, TNF- $\alpha$ , IL-8) and intestinal goblet cell and mast cell numbers in finishing pigs. The results showed that probiotic supplementation had no significant effects on intestinal ITF or TNF- $\alpha$  contents or on mast cell and goblet cell numbers. However, *Bacillus* supplementation tended to decrease IL-8 content in the jejunum, while *Saccharomyces cerevisiae* supplementation tended to decrease IL-8 content in the ileum. Moreover, *Bacillus* supplementation extremely significantly increased SIgA content in both the jejunum and ileum. The results for ITF, IL-8, and SIgA were similar to some recent studies: Wang et al. [56] reported that probiotic supplementation did not affect ITF secretion in rat intestinal tissue; Xiao [57] found that adding probiotics (*Bacillus subtilis* and *Enterococcus faecium*) to weaned piglet diets increased SIgA content in the duodenum and jejunum by 51.5% and 22.5%, respectively; Ren [58] and Jiang [59] reported that feeding lactic acid bacteria to mice downregulated IL-8 transcription levels. Yan et al. [60] reported that probiotics exert anti-inflammatory effects by inhibiting TNF- $\alpha$  activity. The present results contrast with those of Yan et al. [60], possibly due to different bacterial strains used. The results of this study indicate that probiotics can regulate intestinal homeostasis by modulating cytokine secretion, thereby improving immunity.

Dietary supplementation with *Saccharomyces cerevisiae* and *Bacillus* can improve nutrient digestion and absorption in finishing pigs, with *Saccharomyces cerevisiae* showing beneficial effects on intestinal morphological structure, while *Bacillus* can significantly increase SIgA content in the intestine and enhance intestinal immune function. Both *Saccharomyces cerevisiae* and *Bacillus* have the potential to improve intestinal immunity.

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