

Effects of Yeast Culture, *Bacillus subtilis*, and Papain on Growth Performance, Nutrient Apparent Digestibility, and Fecal Microbial Count in Nursery Pigs: Postprint

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Abstract

To investigate the effects of *Saccharomyces cerevisiae* isolated from medicated leaven (MMF) and sourdough, this study examined the impacts of a novel compound yeast culture (YC), *Bacillus subtilis* (BSK), and papain (PE) on growth performance, nutrient apparent digestibility, and fecal microbial counts in nursery pigs. Two hundred twenty-five weaned piglets with an average body weight of (15.70 ± 0.26) kg were selected and randomly divided into 5 groups, with 3 replicates per group and 15 piglets per replicate. The CT group was fed the basal diet + 2×10^{11} CFU/kg BSK, the PE group was fed the basal diet + 0.3 g/kg PE, and the compound (YP) group was fed the basal diet + 5 g/kg YC + 0.3 g/kg PE. The pre-trial period was 5 days, and the formal trial period was 30 days. The results showed: 1) The final weight and average daily gain of the YP group were significantly higher than those of the CT group ($P < 0.05$), and the feed-to-gain ratio was significantly lower than that of the CT group ($P < 0.05$); the final weight of the YC group was higher than that of the CT, BSK, and PE groups ($P > 0.05$). There was no significant difference in average daily feed intake among all groups ($P > 0.05$). The diarrhea rates of the YC, PE, and YP groups were significantly lower than those of the CT and BSK groups ($P < 0.05$). 2) The apparent digestibility of gross energy and dry matter in the YC, BSK, PE, and YP groups was significantly higher than that in the CT group ($P < 0.05$); the apparent digestibility of crude protein and phosphorus in the YC group was significantly lower than that in the YP group ($P < 0.05$); the apparent digestibility of acid detergent fiber and calcium in the YC group was significantly lower than that in the other groups ($P < 0.05$). There was no significant difference in the apparent digestibility of organic matter and crude fat among all groups ($P > 0.05$). 3) The fecal *Lactobacillus* count and *Lactobacillus*/*Escherichia coli* ratio in the YC and YP groups were significantly higher than those in the other groups

($P < 0.05$), and the fecal *Escherichia coli* count was significantly lower than that in the other groups ($P < 0.05$). It can be concluded that the novel YC can improve growth performance and intestinal ecological environment in piglets, and the combination of YC and PE also has positive effects on piglet growth.

Full Text

Effects of Yeast Culture, *Bacillus subtilis* and Papain on Growth Performance, Nutrient Apparent Digestibility and Fecal Microflora Number of Nursery Piglets

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Abstract: To investigate the effects of *Saccharomyces cerevisiae* isolated from *massa medicata fermentata* (MMF) and sourdough, this study examined the impacts of a novel composite yeast culture (YC), *Bacillus subtilis* (BSK), and papain (PE) on growth performance, nutrient apparent digestibility, and fecal microflora number in nursery piglets. A total of 225 weaned piglets with an average body weight of (15.70 ± 0.26) kg were randomly allocated to 5 groups with 3 replicates per group and 15 piglets per replicate. The control (CT) group received the basal diet supplemented with 0.3 CFU/kg BSK, the PE group received the basal diet supplemented with 0.3 g/kg PE, and the complex (YP) group received the basal diet supplemented with 5 g/kg YC plus 0.3 g/kg PE. The pre-trial period lasted 5 days, followed by a 30-day formal trial period. The results showed: 1) The final weight and average daily gain (ADG) of the YP group were significantly higher than those of the CT group ($P < 0.05$), while the feed-to-gain ratio (F/G) was significantly lower ($P < 0.05$). The final weight of the YC group was higher than that of the CT, BSK, and PE groups ($P > 0.05$). No significant differences were observed in average daily feed intake (ADFI) among all groups ($P > 0.05$). The diarrhea rates of the YC, PE, and YP groups were significantly lower than those of the CT and BSK groups ($P < 0.05$). 2) The apparent digestibility of gross energy (GE) and dry matter (DM) in the YC, BSK, PE, and YP groups was significantly higher than in the CT group ($P < 0.05$). The apparent digestibility of crude protein (CP) and phosphorus (P) in the YC group was significantly lower than in the YP group ($P < 0.05$), while the apparent digestibility of neutral detergent fiber (NDF) and calcium (Ca) in the YC group was significantly lower than in all other groups ($P < 0.05$). No significant differences were found in the apparent digestibility of organic matter (OM) and ether extract (EE) among groups ($P > 0.05$). 3) The fecal lactobacilli count and lactobacilli/*Escherichia coli* ratio in the YC and YP groups were significantly higher than in other groups ($P < 0.05$), while fecal *E. coli* counts were significantly lower ($P < 0.05$). These findings indicate that the novel YC can improve piglet growth performance and intestinal micro-ecology, and that the combination of YC and PE also has positive effects on piglet growth.

Keywords: massa medicata fermentata; sourdough; yeast culture; *Bacillus subtilis*; papain; nursery piglets

Yeast culture (YC) is one of the widely used antibiotic alternatives, rich in enzymes, vitamins, proteins, organic acids, carbohydrates, and other nutrients. Numerous studies have demonstrated that dietary YC supplementation can enhance growth performance and immune function while reducing pathogenic bacteria and maintaining intestinal health in ruminants and weaned piglets [1-2]. However, some reports indicate that YC supplementation yields no significant effects on animal growth performance [3]. During YC development, product quality is primarily determined by strain performance [4]. Currently, many yeast strains are available for YC production, but substantial performance differences exist both between species and among strains within the same species, leading to varying effects on rumen fermentation and animal physiology [5]. Therefore, superior yeast strains form the foundation of YC research and application. Massa medicata fermentata (MMF) and sourdough are traditional Chinese naturally fermented preparations that share similarities with modern YC but contain complex microbial communities, including *Saccharomyces cerevisiae*—the fundamental species for YC production. Applying naturally fermented strains to modern YC preparation may open new avenues for microecological agent development.

Bacillus subtilis (BSK) is an ideal feed microbial additive approved for general use in China, characterized by strong resistance to desiccation, high temperature, and high pressure. It secretes highly active lipases, proteases, amylases, pectinases, and cellulases that work synergistically with host digestive enzymes to degrade complex carbohydrates such as xylan, pectin, and cellulose in plant-based feeds, while also breaking down certain anti-nutritional factors to improve nutrient utilization and animal growth performance [6]. Numerous studies have confirmed that BSK can enhance immunity, improve intestinal mucosal morphology, and regulate gut microbial balance in weaned piglets, thereby promoting growth [7-8]. Papain (PE), the earliest discovered and most extensively studied plant protease, can improve nutrient digestibility, particularly protein, degrade anti-nutritional factors such as soybean protein antigens, and enhance animal immunity.

In the emerging field of microecological agents, research has focused on mechanisms and comparative efficacy, with less attention paid to strain selection and performance comparison. BSK is a widely used, high-performance microecological agent, while PE is an excellent plant enzyme for protein hydrolysis. Although YC, BSK, and PE have been frequently applied during the early weaning stage, few studies have investigated their effects on growing nursery pigs. Therefore, this study aimed to evaluate a novel YC developed by our laboratory, comparing it with BSK and PE in nursery pigs, and to assess the effects of these beneficial additives on growth performance, nutrient apparent digestibility, and fecal microflora numbers, providing a theoretical basis for developing new microecological agents.

1.1 Experimental Animals and Grouping

A single-factor randomized block design was employed. A total of 225 healthy “Duroc × Landrace × Large White” crossbred growing pigs with an average body weight of (15.70 ± 0.26) kg were randomly divided into 5 groups based on body weight and sex, with 3 replicates per group. The BSK group received the basal diet supplemented with 0.3 CFU/kg BSK, the PE group received the basal diet supplemented with 0.3 g/kg PE, and the complex (YP) group received the basal diet supplemented with 5 g/kg YC plus 0.3 g/kg PE. The trial consisted of a 5-day pre-test period and a 30-day formal test period.

1.2 Experimental Diets and Management

The basal diet was formulated as a powder according to NRC (1998) standards, with conventional feed ingredients selected based on farm conditions. The composition and nutrient levels are shown in Table 1. The experimental pig house featured a closed, semi-slatted floor design. Pigs followed routine farm procedures for immunization, deworming, disinfection, and management, with ad libitum access to feed and water.

1.3 Experimental Materials

YC was developed by our laboratory as a composite product fermented from two different *Saccharomyces cerevisiae* strains. BSK was provided by Wuhan Huayang Biological Technology Co., Ltd., containing 5×10^{10} CFU/g viable bacteria. PE with an activity of 600,000 U/g was provided by the Biological Research Institute of Guangxi Academy of Sciences.

1.4.1 Growth Performance

On a per-replicate basis, pigs were weighed before morning feeding at the beginning and end of the trial to determine average initial and final weights for calculating average daily gain (ADG). Daily feed allowance was recorded per replicate, with leftover feed quantified weekly to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was then calculated from ADG and ADFI.

1.4.2 Diarrhea Rate

Diarrhea incidence was observed and recorded daily on a per-replicate basis throughout the trial, with diarrhea rate calculated at the conclusion. Diarrhea rate (%) = (total number of diarrheal pigs / total feeding days) × 100.

1.4.3 Fecal Microflora Counts

On day 35 of the trial, fresh fecal samples were collected from 2 randomly selected pigs per replicate and stored at -20 °C for enumeration of *E. coli* and lactobacilli. *E. coli* was cultured on MacConkey agar at 37 °C for 24 h, while

lactobacilli were cultured on MRS medium at 37 °C for 36 h. Microbial counts were expressed as \log_{10} (CFU/g) of feces.

1.4.4 Nutrient Apparent Digestibility

During the final 3 days of the trial, approximately 200 g of uncontaminated fecal samples were collected twice daily from each replicate and stored at -20 °C. Digestibility trials were conducted using 4 mol/L hydrochloric acid-insoluble ash (AIA) as an internal marker to determine DM, gross energy (GE), CP, ether extract (EE), neutral detergent fiber (NDF), organic matter (OM), Ca, P, and AIA content in feed and feces. Nutrient apparent digestibility was calculated as: $\text{Apparent digestibility (\%)} = [1 - (\text{AIA content in feed} / \text{AIA content in feces}) \times (\text{nutrient content in feces} / \text{nutrient content in feed})] \times 100$.

1.5 Data Processing

Data were analyzed using SPSS 19.0 software via one-way ANOVA, with Duncan's multiple comparison test applied. Differences were considered significant at $P < 0.05$.

2.1 Effects of YC, BSK and PE on Growth Performance of Nursery Piglets

As shown in Table 2, the final weight and ADG of the YP group were significantly higher than those of the CT group ($P < 0.05$), while the F/G was significantly lower ($P < 0.05$). The final weight and ADG of the YC group were higher than those of the CT, BSK, and PE groups ($P > 0.05$), indicating a trend toward improved growth performance compared to the CT group. No significant differences were observed in ADFI among groups ($P > 0.05$), though the YP group showed the highest value, followed by the YC group, representing increases of 2.36% and 1.74% over the CT group, respectively. The diarrhea rates of the YC, PE, and YP groups were significantly lower than those of the CT and BSK groups ($P < 0.05$), with no significant differences among the YC, PE, and YP groups themselves ($P > 0.05$).

2.2 Effects of YC, BSK and PE on Nutrient Apparent Digestibility of Nursery Piglets

Table 3 shows that the apparent digestibility of GE and DM in the YC, BSK, PE, and YP groups was significantly higher than in the CT group ($P < 0.05$), with no significant differences among these four groups ($P > 0.05$). The apparent digestibility of CP and P in the YC group was significantly lower than in the YP group ($P < 0.05$) but did not differ significantly from other groups ($P > 0.05$). No significant differences were found in OM and EE apparent digestibility among all groups ($P > 0.05$). The YC group exhibited the lowest apparent digestibility of NDF and Ca, which was significantly lower than all other groups ($P < 0.05$).

2.3 Effects of YC, BSK and PE on Fecal Microflora Number of Nursery Piglets

The fecal lactobacilli counts and lactobacilli/*E. coli* ratio in the YC and YP groups were significantly higher than in other groups ($P < 0.05$), while fecal *E. coli* counts were significantly lower ($P < 0.05$). No significant differences were observed among the other groups ($P > 0.05$).

3.1 Effects of YC, BSK and PE on Growth Performance of Nursery Piglets

YC contains abundant nutrients and growth-promoting factors, with numerous studies confirming its ability to improve weaned piglet growth performance. Mathew et al. [10] and Bontempo et al. [11] demonstrated that dietary YC supplementation enhances ADG and ADFI in weaned piglets. Recently, scientists have developed novel composite YC based on nutritional genomics principles, containing five active substances: glucan, mannan, peptides, organic acids, and amino acids, each with distinct functional effects [12]. Li et al. [13] confirmed that composite YC significantly improves dairy cow growth performance. Our results showed that compared to the CT group, the YC group exhibited non-significant increases in ADG and ADFI (7.20% and 1.74%, respectively) but significantly reduced diarrhea rates, consistent with Van Der Peet-Schwering et al. [14]. This may be attributed to environmental and dietary factors affecting piglet immune function. Van Heugten et al. [15] explained that under high disease stress, YC may trigger intestinal immune responses that interact with the immune system, directing absorbed energy toward immune function rather than growth.

BSK produces substantial amounts of digestive enzymes and nutrients after entering the gastrointestinal tract, enhancing growth performance. Alexopoulos et al. [16] reported that dietary BSK supplementation improves piglet growth performance. In our trial, the BSK group showed no significant differences from the CT group in ADG, ADFI, or F/G, though a positive trend was observed, and diarrhea rates did not differ significantly. This lack of significance may be due to excessive dosage, as Hu et al. [17] found that among different BSK supplementation levels, the 2×10^{10} CFU/kg group showed optimal ADG, ADFI, and diarrhea rates.

Enzyme preparations primarily enhance growth performance by digesting nutrients. Liu et al. [18] found that PE supplementation in piglets weighing approximately 11 kg promoted growth but without significant differences. Bin et al. [19] investigated PE effects on 45- and 75-day-old growing pigs, reporting ADG and feed conversion improvements of 29.60% and 12.54% in the 45-day-old group, and 4.10% and 6.05% in the 75-day-old group, indicating significant growth-promoting effects in 10-20 kg pigs. As piglets age, enzyme effects become less pronounced due to maturing digestive organs and reduced dependence on exogenous enzymes [20]. Our results showed PE had the lowest growth-promoting

effect, with ADG only 5.11% higher than the CT group. These discrepancies with previous studies may stem from differences in enzyme dosage, piglet breed, and rearing environment. Notably, the YP group showed significantly higher final weight and ADG and significantly lower F/G compared to the CT group, outperforming all other groups. Lin [21] reported that combined probiotic and enzyme supplementation in yellow-feathered broilers yielded the highest overall ADG, though ADFI and F/G showed only numerical improvements. Our results also demonstrated that PE and YP groups had significantly lower diarrhea rates than the CT group, possibly due to YC' s immunomodulatory effects or PE' s efficient protein digestion and degradation of soybean antigen proteins in feed. Li et al. [22] showed that soybean antigens can cause allergic diarrhea, intestinal damage, and malabsorption, reducing growth performance, while Kawai et al. [23] and Shutov et al. [24] confirmed PE' s effectiveness in degrading soybean antigen proteins.

3.2 Effects of YC, BSK and PE on Nutrient Apparent Digestibility of Nursery Piglets

MMF is a traditional Chinese medicine used to improve gastrointestinal digestion, while sourdough is used in traditional rice wine, yellow wine, and rice cake production. Studies indicate that yeast serves as the primary fermentation agent in both MMF and sourdough [25-26]. Pinloche et al. [27] reported that YC supplementation in growing pig diets stimulates hindgut fermentation, increasing volatile fatty acid production and bacterial fermentation end products, thereby improving nutrient apparent utilization. Shen et al. [28] found that 5 g/kg YC supplementation in weaned piglets significantly improved apparent digestibility of CP, DM, and GE. Our results showed that the YC group had significantly higher GE and DM apparent digestibility than the CT group, but lower OM, CP, EE, and P digestibility, with NDF and Ca digestibility significantly lower than all other groups. These findings partially align with but also differ from previous studies, likely due to variations in microbial agents, diets, and breeds. BSK secretes highly active lipases, proteases, amylases, and cellulases that facilitate nutrient digestion, while PE is an excellent protein-degrading enzyme that enhances endogenous protease activity and promotes protein digestion and absorption. Our results showed that BSK and PE groups had significantly higher GE and DM apparent digestibility than the CT group, with other nutrient digestibility values slightly higher than the YC group but not significantly different from the CT group. Noh et al. [29] reported that 5% BSK supplementation significantly improved apparent digestibility of DM, GE, and crude ash. Xuan et al. [30] found that compound enzyme supplementation in weaned piglets numerically improved all nutrient apparent digestibility values, with P digestibility significantly increased. Differences from our study may be due to excessive probiotic counts producing surplus enzymes that inhibit endogenous lipase and phosphorus metabolic enzyme activities and secretion, thereby reducing overall nutrient degradation [31]. Although the YC and PE combination showed no significant differences in nutrient apparent digestibility, numerical

values were higher than other groups.

3.3 Effects of YC, BSK and PE on Fecal Microflora Number of Nursery Piglets

The primary objective of microecological agents is to implant exogenous probiotics or improve endogenous microflora to promote growth. Xie et al. [32] demonstrated that 5% or 10% YC supplementation in vitro significantly promoted proliferation of three beneficial intestinal bacteria: *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Bifidobacterium*. Our results showed that compared to the CT group, YC and YP groups significantly reduced fecal *E. coli* counts while increasing lactobacilli counts and the lactobacilli/*E. coli* ratio, consistent with Upadrasta et al. [33]. This may be related to diet-induced stress, as yeast polysaccharides in YC inhibit pathogen colonization while providing nutrients that fuel beneficial bacterial proliferation, thereby improving gastrointestinal environment [34]. However, the lack of significant growth performance improvement in the YC group validates Scheuermann's [35] conclusion that improved gut microbial balance does not necessarily translate to enhanced growth performance.

Our PE group showed decreased lactobacilli and *E. coli* counts without altering the lactobacilli/*E. coli* ratio, indicating no significant effect on gut microflora. As a protease, PE's primary function may be protein digestion and utilization. Yin [36] reported that 0.03% non-starch polysaccharide enzyme complex significantly inhibited intestinal *E. coli* growth, suggesting that different enzymes produce varying effects depending on test animal breed and inherent gut microbial differences. *Bacillus* additives primarily function by regulating animal microbial balance. Su et al. [37] found that *Bacillus* increased the lactobacilli/*E. coli* ratio in jejunal and colonic digesta of post-weaning piglets, while Chen et al. [38] reported that dietary *Bacillus* supplementation significantly increased *Bifidobacterium* and lactobacilli counts while reducing *E. coli* in growing-finishing pigs. In contrast, our BSK group showed no significant differences from the CT group in *E. coli* counts or lactobacilli/*E. coli* ratio but significantly reduced lactobacilli numbers, suggesting a relationship between beneficial bacteria counts and diarrhea rates. The reduction in lactobacilli by BSK may be dose-related, requiring further gut microbial analysis to validate this conclusion.

In conclusion, this novel YC can improve piglet growth performance and intestinal micro-ecology, and the combination of YC and PE also demonstrates positive effects on piglet growth.

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