Effects of Yeast Cell Wall Polysaccharides on Intestinal Volatile Fatty Acids and Microbial Flora in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of dietary yeast cell wall polysaccharide supplementation on intestinal volatile fatty acids and microbial flora in weaned piglets. A single-factor experimental design was adopted, and 180 21-day-old weaned piglets with consistent genetic background, good health status, similar parity and body weight were selected and randomly divided into 4 groups, with 5 replicates per group and 9 piglets per replicate. The pigs in the four groups were fed a control diet (without yeast cell wall polysaccharide), 0.15% yeast cell wall polysaccharide diet, 0.30% yeast cell wall polysaccharide diet, and 0.45% yeast cell wall polysaccharide diet, respectively. The experimental period was 21 days. The results showed that: 1) Compared with the control group, dietary supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharide significantly increased the content of acetate in the colon of piglets ($P<0.05$); among them, 0.30% and 0.45% yeast cell wall polysaccharide also significantly increased the contents of propionate, butyrate, and valerate as well as total volatile fatty acids in the colon ($P<0.05$), with no significant difference between the two groups ($P>0.05$). 2) Compared with the control group, 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharide significantly reduced the numbers of Salmonella and Escherichia coli in the cecum ($P<0.05$), with no significant difference between the 0.30% and 0.45% yeast cell wall polysaccharide groups ($P>0.05$). It can be concluded that yeast cell wall polysaccharide can increase the content of intestinal volatile fatty acids and improve the structure of intestinal microbial flora in piglets. According to regression equation prediction, the optimal supplementation level of yeast cell wall polysaccharide in piglet diets is 0.31%~0.40%.

Full Text

Effects of Yeast Cell Wall Polysaccharides on Intestinal Volatile Fatty Acids and Microbial Flora of Weaned Piglets

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Abstract

This study investigated the effects of dietary yeast cell wall polysaccharides on intestinal volatile fatty acids (VFA) and microbial flora in weaned piglets. Using a single-factor experimental design, 180 healthy 21-day-old weaned piglets with consistent genetic background, similar parity and body weight were randomly allocated to 4 groups with 5 replicates each (9 piglets per replicate). The four groups were fed either a control diet (without yeast cell wall polysaccharides) or diets supplemented with 0.15%, 0.30%, or 0.45% yeast cell wall polysaccharides for a 21-day experimental period. The results showed that: (1) Compared with the control group, dietary supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides significantly increased colonic acetic acid content ($P<0.05$). The 0.30% and 0.45% supplementation levels also significantly elevated colonic propionic acid, butyric acid, valeric acid, and total volatile fatty acid (TVFA) contents ($P<0.05$), with no significant differences between these two groups ($P>0.05$). (2) Supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides significantly reduced cecal Salmonella and Escherichia coli populations compared to the control group ($P<0.05$), while no significant difference was observed between the 0.30% and 0.45% groups ($P>0.05$). These findings demonstrate that yeast cell wall polysaccharides can increase intestinal VFA content and improve intestinal microbial flora structure in weaned piglets. Based on regression equation predictions, the optimal dietary inclusion level of yeast cell wall polysaccharides for weaned piglets is 0.31%-0.40%.

Keywords: yeast cell wall polysaccharide; weaned piglets; volatile fatty acids; intestinal flora

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Introduction

Modern pig production has become increasingly technology- and capital-intensive, facing the dual challenge of providing high-quality pork while safeguarding environmental hygiene and human health. The misuse and overuse of antibiotic feed additives have exacerbated problems including antibiotic residues, bacterial multidrug resistance, and environmental pollution

[1-2], creating an urgent need for suitable antibiotic alternatives. Yeast cell wall polysaccharides, functional oligosaccharides composed primarily of β-glucan and mannan oligosaccharides, have shown promise in this regard. Numerous studies have demonstrated that oligosaccharides can improve gut microbial community structure and enhance immune function [3-5]. Similarly, yeast cell wall polysaccharides have been proven to improve growth performance, immune function, and intestinal health in livestock and poultry without harmful residues [6]. However, the specific mechanisms through which they improve intestinal health and their effects on gut microbiota in weaned piglets require further investigation.

Volatile fatty acids (VFA) are important intermediate products of microbial anaerobic fermentation. The hindgut represents the most microbiologically rich region of the piglet intestinal tract, with the colon being the primary site of VFA production in monogastric animals. This is because bacteria in the colon first encounter undigested complex carbohydrates from the small intestine, resulting in the strongest fermentation activity. Additionally, the colon is longer than the cecum and contains relatively more microorganisms [7-8]. This experiment was designed to investigate the effects of yeast cell wall polysaccharides on intestinal VFA and microbial flora in weaned piglets, determine the optimal supplementation level, and provide a theoretical basis for the scientific application of yeast cell wall polysaccharides in piglet diets.

Materials and Methods

1.1 Experimental Material Yeast cell wall polysaccharides were obtained from Top Bio-Technology Co., Ltd., with main components including mannan oligosaccharides (23.45%) and β-glucan (39.24%), along with 26.30% crude protein, 3.30% crude ash, and 5.35% moisture.

1.2 Experimental Animals and Design One hundred eighty 21-day-old weaned piglets with uniform genetic background, good health status, and similar parity and body weight were selected and randomly allocated to 4 groups using a single-factor completely randomized block design. Each group contained 5 replicates with 9 piglets per replicate. The four dietary treatments consisted of a control diet (without yeast cell wall polysaccharides) and diets supplemented with 0.15%, 0.30%, or 0.45% yeast cell wall polysaccharides. The experimental period lasted 21 days.

1.3 Experimental Diets and Nutrient Levels Piglets were fed corn-soybean meal-based diets formulated according to NRC (2012) and Chinese Feeding Standard of Swine (NY/T 65−2004). Diet composition and nutrient levels are presented in Table 1 .

Table 1 Diet composition and nutrient levels (air-dry basis), %

[Note: The table content would be preserved here with proper formatting, showing ingredients and nutrient levels across the four treatment groups]

1.4 Animal Management Piglets were housed in nursery pens equipped with elevated slatted floors and nipple drinkers. The pig house was thoroughly cleaned and disinfected before the experiment. During the trial, piglets were fed 4–5 times daily with ad libitum access to feed and water. Other management practices and immunization programs followed conventional farm procedures.

1.5 Sample Collection and Processing On day 21 of the experiment, one piglet near the average body weight and in good health was selected from each replicate, anesthetized via intramuscular injection of 4% sodium pentobarbital solution, and euthanized by exsanguination via the jugular vein after complete anesthesia. The abdominal cavity was opened, and the colon was quickly isolated. Colonic segments with digesta were excised, with both ends ligated and fixed, then immediately frozen in liquid nitrogen for subsequent VFA analysis.

Additionally, the middle segment of the cecum was isolated, double-ligated at both ends, wrapped in aluminum foil and plastic wrap with cotton thread, labeled, rapidly frozen in liquid nitrogen, and stored at -80°C for later analysis.

1.6 Analytical Methods

1.6.1 Determination of Colonic VFA Samples were thawed and processed according to the method of Geng et al. [9]. Briefly, 1.0 g of colonic content was accurately weighed into an EP tube, mixed with 1 mL ultrapure water, and vortexed until homogeneous. The mixture was centrifuged at 15,000 r/min for 15 minutes, and the supernatant was transferred and treated with 25% metaphosphoric acid at a 9:1 volume ratio in an ice bath for 3 hours. Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid contents were then determined by gas chromatography using external standard methods, and total volatile fatty acid (TVFA) content was calculated.

1.6.2 Cecal Bacterial Counting Cecal E. coli, Salmonella, Bifidobacterium, Lactobacilli, and total bacteria were enumerated using plate culture methods. Cecal content samples were thawed at room temperature in a sterile workstation. Approximately 0.5 g of cecal content was weighed into a sterile penicillin bottle, mixed with 5 mL sterile physiological saline, and vortexed for 3–5 minutes. A 500 L aliquot of the diluted solution was then transferred to another sterile penicillin bottle containing 4.5 mL physiological saline, vortexed for 1–2 minutes to create a 10^{-1} dilution, and serial dilutions of 10^{-2} to 10^{-6} were prepared by repeating this process.

The diluted cecal content was inoculated onto appropriate culture media plates. Eosin methylene blue, MRS, HE, BBL, and standard media were used for enumerating E. coli, Lactobacilli, Salmonella, Bifidobacterium, and total bacteria,

respectively. Five dilution gradients were tested for each parameter with three replicates per gradient. Total bacteria, E. coli, and Salmonella were cultured aerobically at 37°C for 24 hours, while Bifidobacterium and Lactobacilli were cultured anaerobically at 37°C for 48 hours. Plates were then removed for colony counting, with results expressed as $\log_{10}$ colony-forming units per gram of digesta [lg(CFU/g)].

1.7 Data Processing and Statistical Analysis All data were initially processed using Excel 2003 and then subjected to one-way ANOVA using SPSS 17.0 software. When significant differences were detected, Duncan' s multiple comparison test was applied. Curvilinear regression analysis was performed using SPSS 17.0 to establish the relationship between dietary yeast cell wall polysaccharide levels and various parameters through quadratic curve fitting, thereby determining the optimal supplementation level. Data are presented as "mean ± standard error," with $P<0.05$ considered statistically significant.

Results

2.1 Effects of Yeast Cell Wall Polysaccharides on Colonic VFA in Weaned Piglets As shown in Table 2 , compared with the control group, dietary supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides increased colonic acetic acid content by 27.57%, 48.71%, and 34.46%, respectively ($P<0.05$). Supplementation with 0.30% and 0.45% yeast cell wall polysaccharides also significantly elevated colonic propionic acid, butyric acid, and valeric acid contents ($P<0.05$). Overall, dietary supplementation with 0.30% and 0.45% yeast cell wall polysaccharides significantly increased colonic TVFA content ($P<0.05$), with increases of 49.72% and 44.42% compared to the control group, respectively ($P<0.05$). However, no significant differences were observed between the 0.30% and 0.45% groups for propionic acid, butyric acid, valeric acid, or TVFA contents ($P>0.05$).

Curve-fitting regression analysis revealed quadratic relationships between dietary yeast cell wall polysaccharide supplementation level (X) and both acetic acid (Y_1) and TVFA (Y_2) contents in the colon, with the following regression equations:

$Y_1 = -7{,}513.000X^2 + 4{,}723.203X + 1{,}593.519$ ($R^2 = 0.677$, $P = 0.006$)
$Y_2 = -9{,}497.699X^2 + 7{,}419.340X + 2{,}909.547$ ($R^2 = 0.727$, $P = 0.003$)

Based on these equations, maximal colonic acetic acid content was achieved at a dietary yeast cell wall polysaccharide level of 0.31%, while maximal TVFA content was attained at 0.39% supplementation.

Table 2 Effects of yeast cell wall polysaccharides on volatile fatty acids in colon of weaned piglets (g/mL)

[Table content showing the effects on acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, and TVFA across treatment groups, with statistical notation]

2.2 Effects of Yeast Cell Wall Polysaccharides on Cecal Microbial Flora in Weaned Piglets As presented in Table 3 , no significant differences were observed among groups for cecal Bifidobacterium, Lactobacilli, or total bacterial populations (P>0.05). However, dietary supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides reduced cecal Salmonella counts by 6.12%, 7.93%, and 9.09% compared to the control group (P<0.05). Additionally, yeast cell wall polysaccharide supplementation significantly decreased intestinal E. coli populations (P<0.05).

Regression analysis revealed a quadratic relationship between cecal Salmonella counts (Y_3) and dietary yeast cell wall polysaccharide level (X), described by the equation:

$Y_3 = 3.259X^2 - 2.636X + 6.036$ ($R^2 = 0.670$, $P = 0.007$)

This equation indicates that the minimal cecal Salmonella population occurs at a dietary yeast cell wall polysaccharide supplementation level of 0.40%.

Table 3 Effects of yeast cell wall polysaccharides on cecal bacterial counts in weaned piglets [lg(CFU/g)]

[Table content showing bacterial counts for E. coli, Salmonella, Bifidobacterium, Lactobacilli, and total bacteria across treatment groups]

Discussion

3.1 Effects of Yeast Cell Wall Polysaccharides on Colonic VFA in Weaned Piglets The large intestine contains the highest microbial density throughout the digestive tract of monogastric animals [10]. Although microbial endotoxins can be harmful to the intestine, these microorganisms maintain intestinal microecological balance, resist colonization by foreign microbes, and activate the intestinal immune system [11]. More importantly, they ferment complex carbohydrates into VFA [12-13]. Both in vivo and in vitro studies have confirmed that oligosaccharides can be degraded by bacteria into substantial amounts of VFA [14-15]. The present results demonstrate that supplementation with 0.30% and 0.45% yeast cell wall polysaccharides significantly increased TVFA content in weaned piglets. Pan [16] reported that mannan oligosaccharides significantly increased TVFA, propionic acid, and valeric acid contents in the cecum of mice. Hang et al. [15] investigated the effects of mannan oligosaccharides and beet juice on intestinal microbial fermentation in vitro, finding that mannan oligosaccharide groups showed significantly higher proportions of propionic and butyric acids in the ileum compared to the control group, though acetic acid proportion was significantly lower. In contrast, our results show that

yeast cell wall polysaccharides significantly increased acetic, propionic, and butyric acid contents. Microorganisms in the large intestine can convert lactate and acetate into butyrate [17], and complete lactate fermentation can produce substantial propionic acid. Therefore, we speculate that yeast cell wall polysaccharides may promote the proliferation of lactate-utilizing bacteria to facilitate the conversion of lactate to butyrate. Additionally, our study found that 0.45% yeast cell wall polysaccharide supplementation significantly increased colonic valeric acid content. Through regression analysis of the relationship between dietary yeast cell wall polysaccharide level and colonic TVFA and acetic acid contents, we determined that VFA content was highest when dietary yeast cell wall polysaccharide levels were 0.39% and 0.31%, respectively.

3.2 Effects of Yeast Cell Wall Polysaccharides on Cecal Microbial Flora in Weaned Piglets Under normal conditions, various microbial populations in the animal intestine maintain a dynamic equilibrium through mutual dependence and antagonism. However, environmental stressors such as weaning, temperature changes, and pen transfer can disrupt this balance, leading to dysbiosis. Research has confirmed that after weaning, intestinal Lactobacilli populations decline while E. coli proportions increase [18-19]. Although antibiotics can improve piglet diarrhea to some extent, they also destroy beneficial intestinal bacteria [20]. Yeast cell wall polysaccharides, primarily containing mannan oligosaccharides and β-glucan, can influence intestinal microbial ecology [21-22]. Over two decades ago, functional oligosaccharides such as mannan oligosaccharides and fructooligosaccharides were shown to improve human intestinal flora [23]. Previous animal studies have also demonstrated that supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides significantly increased average daily gain and average daily feed intake in weaned piglets, with a trend toward reduced feed conversion ratio and diarrhea rate [24].

Our results show that dietary yeast cell wall polysaccharides significantly reduced cecal Salmonella and E. coli populations in weaned piglets. This may occur because mannan oligosaccharides can be utilized and fermented by intestinal microbes, promoting VFA production. On one hand, increased VFA lowers intestinal pH, thereby inhibiting the growth of acid-sensitive harmful bacteria such as E. coli and Salmonella [25]. On the other hand, VFA can reduce redox potential, affecting the oxidation-reduction reactions of essential coenzymes required for the growth and metabolism of harmful bacteria [26], thus suppressing pathogenic organisms. Sa [27] reported that mannan oligosaccharides significantly reduced intestinal E. coli counts in weaned piglets. Additionally, studies have found that mannan oligosaccharides can enhance resistance to Salmonella and E. coli infections in weaned piglets [28]. β-glucan also modulates intestinal microbes, with supplementation increasing beneficial bacterial populations such as Lactobacilli and Bifidobacterium [29], consistent with our findings. Although not statistically significant, our study observed that supplementation with 0.15%, 0.30%, and 0.45% yeast cell wall polysaccharides tended to increase cecal Bifidobacterium and Lactobacilli populations compared

to the control group. Collectively, these results demonstrate that yeast cell wall polysaccharides can improve intestinal flora structure by promoting the proliferation of beneficial bacteria while inhibiting harmful bacteria. Regression analysis of cecal Salmonella counts and dietary yeast cell wall polysaccharide level revealed optimal effects at a supplementation level of 0.40%.

Conclusions

1. Yeast cell wall polysaccharides can modulate the composition and content of intestinal VFA in piglets. Supplementation with 0.30% and 0.45% yeast cell wall polysaccharides significantly increased colonic contents of acetic acid, propionic acid, butyric acid, valeric acid, and TVFA.
2. Yeast cell wall polysaccharides promote improved intestinal flora structure in weaned piglets. Dietary supplementation with 0.30% and 0.45% yeast cell wall polysaccharides significantly reduced cecal E. coli and Salmonella populations.
3. Regression equation predictions indicate that the optimal dietary inclusion level of yeast cell wall polysaccharides for piglets is 0.31%–0.40%.

References

[1] ZHAO Q, ZHONG T M, HU Y M. Effects of veterinary drug and feed additive residues on human health[J]. Chinese Journal of Animal Science, 2005, 41(1): 55–57.
[2] GONG X, CHENG X H. Research on antimicrobial resistance of feed antibiotics: present and future[J]. Chinese Journal of Animal Science, 2007, 43(22): 26–32.
[3] HUANG X W, YANG S S, LIU Y, et al. Effects of chitosan oligosaccharide on growth performance, lipid metabolism, non-specific immune function and intestinal health of juvenile Jian carp[J]. Chinese Journal of Animal Nutrition, 2015, 27(7): 2106–2114.
[4] LI M Y, ZHU K Y, LIU Y H, et al. Effects of dietary fructooligosaccharide supplementation on performance, serum indices, and fecal pH, microbial flora and volatile fatty acid contents in primiparous sows[J]. Chinese Journal of Animal Nutrition, 2015, 27(2): 510–516.
[5] GUO F, SUN Y N, ZHOU Y M, et al. Effects of compound oligosaccharides on performance and immune organ indices of broilers[J]. Journal of Domestic Animal Ecology, 2009, 30(4): 25–28.
[6] ZHOU X, ZHANG S S, LI L, et al. Research progress on physiological functions of yeast polysaccharides and their application in animal feeding[J]. Cereal & Feed Industry, 2013(10): 46–48.
[7] LIAN X W. Study on short-chain fatty acid production from several dietary

fibers by intestinal microbiota in vitro[D]. Master' s thesis. Guangzhou: Jinan University, 2011.
[8] LI C H, MENG X Q, LIU B T, et al. Effects of activated ovalbumin on SCFA in colon of weaned piglets[J]. Acta Agriculturae Zhejiangensis, 2014(2): 297-302.
[9] GENG M M, XU L W, YUAN H C, et al. Determination of short-chain fatty acids in pig colon contents by gas chromatography[J]. Progress in Modern Biomedicine, 2015, 15(6): 1010-1014.
[10] JENSEN B B, JØRGENSEN H. Effect of dietary fiber on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs[J]. Applied and Environmental Microbiology, 1994, 60(6): 1897-1904.
[11] ZHOU M Y. Study on the regulatory effect of salecan on intestinal physiology and pathology[D]. Doctoral dissertation. Nanjing: Nanjing University of Science and Technology, 2014.
[12] WANG L F. Study on the effects of probiotic L. plantarum P-8 on intestinal flora, intestinal immunity and growth performance in broilers[D]. Doctoral dissertation. Hohhot: Inner Mongolia Agricultural University, 2014.
[13] SAMUEL B S, SHAITO A, MOTOIKE T, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41[J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(43): 16767-16772.
[14] CUMMINGS J H, MACFARLANE G T, ENGLYST H N. Prebiotic digestion and fermentation[J]. American Journal of Clinical Nutrition, 2001, 73(S2): 415S-420S.
[15] HANG S Q, MAO S Y, YU Z T, et al. In vitro evaluation of effects of mannan oligosaccharides and beet juice on intestinal microbial fermentation[J]. Journal of Nanjing Agricultural University, 2007, 30(1): 79-83.
[16] PAN X D. Studies on functional properties of several oligosaccharides and their regulatory mechanisms on intestinal physiology and ecology[D]. Doctoral dissertation. Hangzhou: Zhejiang University, 2009.
[17] DUNCAN S H, HOLTROP G, LOBLEY G E, et al. Contribution of acetate to butyrate formation by human faecal bacteria[J]. British Journal of Nutrition, 2004, 91(6): 915-923.
[18] CANH T T, SUTTON A L, AARNINK A J, et al. Dietary carbohydrates alter the fecal composition and pH and the ammonia emission from slurry of growing pigs[J]. Journal of Animal Science, 1998, 76(7): 1887-1895.
[19] MATHEW A G, SUTTON A L, SCHEIDT A B, et al. Effect of galactan on selected microbial populations and pH and volatile fatty acids in the ileum of the weanling pig[J]. Journal of Animal Science, 1993, 71(6): 1503-1509.
[20] BLAKE D P, HILLMAN K, FENLON D R. The use of a model ileum to investigate the effects of novel and existing antimicrobials on indigenous porcine gastrointestinal microflora: using vancomycin as an example[J]. Animal Feed Science and Technology, 2003, 103(1/2/3/4): 123-139.
[21] SHANMUGASUNDARAM R, SIFRI M, SELVARAJ R K. Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection[J]. Poultry

Science, 2013, 92(2): 358–363.
[22] SHANMUGASUNDARAM R, SIFRI M, SELVARAJ R K. Effect of yeast cell product supplementation on broiler cecal microflora species and immune responses during an experimental coccidial infection[J]. Poultry Science, 2013, 92(5): 1195–1201.
[23] TANAKA R, TAKAYAMA H, MOROTOMI M, et al. Effects of Administration of TOS and Bifidobacterium breve on human fecal flora[J]. Bifidobacteria Microflora, 1983, 2(1): 17–24.
[24] HE Q, WANG Z R, YOU J M, et al. Effects of yeast cell wall polysaccharides on growth performance and small intestinal mucosal morphology in weaned piglets[J]. Chinese Journal of Animal Nutrition, 2016, 28(11): 3536–3541.
[25] HANG S Q. Effects of mannan oligosaccharides on intestinal microbes in weaned piglets[D]. Doctoral dissertation. Nanjing: Nanjing Agricultural University, 2007.
[26] WU Y Y, GUO Y M, WANG Z, et al. Effects of xylooligosaccharides on growth performance, intestinal physiology and morphology in broilers[J]. Journal of China Agricultural University, 2006, 11(4): 42–46.
[27] SA L F, BIAN L Q, PAN S D. Effects of mannan oligosaccharides on growth performance and diarrhea rate in weaned piglets[J]. Animal Science and Animal Medicine, 2005, 22(10): 46–47.
[28] WHITE L A, NEWMAN M C, CROMWELL G L, et al. Brewers dried yeast as a source of mannan oligosaccharides for weanling pigs[J]. Journal of Animal Science, 2002, 80(10): 2619–2628.
[29] PAN S D, LI X J, BIAN L Q, et al. Effects of yeast β-glucan on intestinal flora in weaned piglets[J]. Feed Industry, 2012, 33(12): 21–23.

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