Effects of Yeast Hydrolysate Combined with Complex Enzyme Preparation or Microecological Preparation as a Replacement for Spray-Dried Plasma Protein on Growth Performance and Serum Biochemical Indices in Nursery Pigs (Postprint)

Authors: Wen Chaoyue, Li Yong, Xing Weigang, Li Biao, Liu Jia, Ma Xiangdong, Zhang Yuzhe, Li Fengna

Date: 2017-10-11T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of combined application of yeast hydrolysate with compound enzyme preparation or microecological preparation on growth performance and serum biochemical indices of weaned piglets. A total of 480 healthy 35-day-old nursery pigs with similar body condition and body weight from "Duroc × Landrace × Yorkshire" three-way crossbred were selected and randomly divided into 5 groups with a 1:1 sex ratio, 8 replicates per group and 12 pigs per replicate. A single-factor experimental design was adopted. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 2% plasma protein powder (Group 1), 0.5% yeast hydrolysate (Group 2), 0.5% yeast hydrolysate + 0.02% compound enzyme preparation (Group 3), and 0.5% yeast hydrolysate + 0.03% microecological preparation (Group 4), respectively. The experiment included a 3-day pre-trial period followed by a 25-day formal trial period, which was divided into two phases: Phase I (days 1–14) and Phase II (days 15–25). The results showed that: 1) During Phase I, the average daily gain (ADG) and average daily feed intake (ADFI) of nursery pigs in all experimental groups were significantly higher than those in the control group ($P<0.05$), and the feed to gain ratio (F/G) of Group 3 was significantly lower than that of the control group ($P<0.05$), while the values of the remaining groups were intermediate. During Phase II, no significant differences were observed in ADG and F/G among groups ($P>0.05$); however, compared with the control group, the ADFI of nursery pigs in Group 1 was significantly increased ($P<0.05$), and the diarrhea rates of Groups 1, 2, and

4 were significantly lower than those of the control group and Group 3 (P<0.05). Over the entire trial period, compared with the control group, the ADG and ADFI of nursery pigs in Group 1 were significantly increased (P<0.05), while the values of the other groups were intermediate. 2) Compared with Group 2, the serum triglyceride content of nursery pigs in Group 3 was significantly decreased (P<0.05), while no significant differences were observed among groups for other serum biochemical indices (P>0.05). It can be concluded that plasma protein powder and yeast hydrolysate can improve the growth performance of nursery pigs to varying degrees, yeast hydrolysate has the potential to replace plasma protein powder in nursery pig diets, but the combined application of yeast hydrolysate with microecological preparation or compound enzyme preparation did not produce synergistic effects.

Full Text

Effects of Combined Application of Yeast Hydrolysate and Multi-Enzyme Preparations or Probiotics as a Substitute for Plasma Protein Powder on Growth Performance and Serum Biochemical Indices of Nursery Piglets

WEN Chaoyue[1,2], LI Yong[3], XING Weigang[3], LI Biao[4], LIU Jia[3], MA Xiangdong[5], ZHANG Yuzhe[1], LI Fengna[1,6]*

[1]Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China;
[2]Laboratory of Animal Nutrition and Human Health, School of Life Sciences, Hunan Normal University, Changsha 410006, China;
[3]New Hope Liuhe Co., Ltd., Beijing 100102, China;
[4]Angel Yeast Co., Ltd., Yichang 443003, China;
[5]Qingdao Vland Biotech Co., Ltd., Qingdao 266001, China;
[6]Hunan Co-Innovation Center of Animal Production Safety, Changsha 410128, China

Abstract: This experiment aimed to investigate the effects of combined application of yeast hydrolysate with multi-enzyme preparations or probiotics on growth performance and serum biochemical indices of weaned piglets. Four hundred and eighty healthy 35-day-old crossbred (Duroc × Landrace × Yorkshire) nursery piglets with similar body weight were randomly assigned to 5 groups, with 8 replicates per group and 12 piglets per pen (balanced for gender). Using a single-factor experimental design, the control group received a basal diet, while treatment groups received the basal diet supplemented with 2% plasma protein powder (Group 1), 0.5% yeast hydrolysate (Group 2), 0.5% yeast hydrolysate + 0.02% multi-enzyme preparations (Group 3), and 0.5% yeast hydrolysate + 0.03% probiotics (Group 4), respectively. The trial consisted of a 3-day adaptation period followed by a 25-day formal experimental period, divided into Stage

I (days 1–14) and Stage II (days 15–25). The results showed: 1) In Stage I, average daily gain (ADG) and average daily feed intake (ADFI) of nursery pigs in all treatment groups were significantly higher than those in the control group ($P<0.05$), and the feed-to-gain ratio (F/G) in Group 3 was significantly lower than that in the control group ($P<0.05$), while values for remaining groups were intermediate. In Stage II, no significant differences were observed among groups in ADG and F/G ($P>0.05$); however, compared with the control group, ADFI in Group 1 was significantly increased ($P<0.05$), and diarrhea incidence in Groups 1, 2, and 4 was significantly lower than that in the control group and Group 3 ($P<0.05$). Over the entire experimental period, ADG and ADFI in Group 1 were significantly higher than those in the control group ($P<0.05$), while values for other groups were intermediate. 2) Serum triglyceride content in Group 3 was significantly lower than that in Group 2 ($P<0.05$), while no significant differences were detected among groups for other serum biochemical indices ($P>0.05$). These findings indicate that both plasma protein powder and yeast hydrolysate can improve growth performance of nursery piglets to varying degrees, and that yeast hydrolysate has potential to replace plasma protein powder in nursery pig diets. However, combined application of yeast hydrolysate with probiotics or multi-enzyme preparations did not produce synergistic effects.

Key words: yeast hydrolysate; multi-enzyme preparation; probiotics; growth performance; serum biochemical index; nursery piglet

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Plasma protein powder is widely used in piglet diets due to its rich nutritional value; however, its biosafety has been increasingly questioned, making the search for alternatives increasingly important. Yeast hydrolysate is rich in amino acids, nucleotides, small peptides, and mannan-oligosaccharides[1], which can improve growth performance of weaned piglets, promote intestinal development[2], and reduce diarrhea incidence[3]. Multi-enzyme preparations can compensate for insufficient endogenous digestive enzyme secretion, reduce piglet diarrhea caused by indigestion, improve nutrient digestibility, and thereby enhance growth performance[4-5]. Probiotics can regulate intestinal microecological environment, reduce diarrhea incidence, and thus promote piglet growth[6]. Evidently, all three additives can promote piglet growth through different mechanisms. However, whether combined application of yeast hydrolysate with multi-enzyme preparations or probiotics can replace plasma protein powder and produce synergistic effects has not been reported. Therefore, this experiment used nursery piglets as experimental subjects to compare feeding effects of different nutritional interventions and to preliminarily evaluate piglet growth performance and serum biochemical indices.

1.1 Experimental Materials

Yeast hydrolysate (Angel Yeast NA100) contained >35% crude protein (CP). The multi-enzyme preparation (Qingdao Vland) primarily contained protease,

non-starch polysaccharide enzymes, xylanase, and β-glucanase. The probiotic preparation (Qingdao Vland) mainly contained *Bacillus subtilis*, *Enterococcus faecium*, and other strains, with a viable count of 2$×10^{10}$ CFU/g.

1.2 Animals and Grouping

The experiment utilized 480 healthy 35-day-old crossbred (Duroc × Landrace × Yorkshire) nursery piglets with an initial body weight of (11.71$±$0.93) kg. Using a single-factor experimental design, piglets were randomly allocated to 5 groups according to the principle of similar body weight and genetic background, with 8 replicates per group and 12 piglets per replicate pen, maintaining a 1:1 gender ratio. Each group was randomly assigned to one of the experimental diets. The trial included a 3-day adaptation period followed by a 25-day formal experimental period, which was divided into Stage I (days 1–14) and Stage II (days 15–25).

1.3 Diets and Management

Five dietary formulations were designed according to nutrient requirements recommended by NRC (2012) and the ideal amino acid pattern for pigs, using a standardized ileal digestible amino acid system. The experimental grouping is shown in Table 1 , and dietary composition and nutrient levels are presented in Table 2 . The experiment was conducted in a fully enclosed nursery pig house with constant temperature of 26°C and relative humidity of 65%–75%. The facility featured slatted plastic flooring, stainless steel adjustable feeders, and nipple drinkers. Metabolic cages and floors were cleaned daily, and the pig house was disinfected by spraying once weekly. Routine management procedures for deworming and vaccination were followed, and the health status of pigs was observed and recorded daily.

1.4.1 Growth Performance Measurement

Body weight was measured at 08:00 on the first day, day 15, and day 25 of the formal experimental period after overnight fasting. Daily feed provision, feed loss, and feed residue for each group were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G). The number of piglets with diarrhea was recorded to calculate diarrhea incidence.

1.4.2 Serum Biochemical Indices Measurement

On the final day of Stage II, 6 pigs were randomly selected from each group and 5 mL of blood was collected from the anterior vena cava at 08:00 after overnight fasting. The blood was placed at an angle to allow serum separation, then centrifuged at 3,000 r/min for 15 minutes. Serum was collected, aliquoted into EP tubes, and stored at -20°C. Serum biochemical indices were measured using a Shenzhen Mindray BS-190 automatic biochemical analyzer, with reagent kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis

Experimental data were analyzed using the one-way ANOVA module in SPSS 22.0 statistical software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means and standard errors. $P<0.05$ was considered statistically significant, while $P>0.05$ indicated no significant difference.

2.1 Effects of Combined Application of Yeast Hydrolysate and Multi-Enzyme Preparations or Probiotics on Growth Performance and Diarrhea Rate of Nursery Piglets

As shown in Table 3 , during Stage I, ADG and ADFI in all treatment groups were significantly higher than those in the control group ($P<0.05$). The combination of yeast hydrolysate and multi-enzyme preparations (Group 3) significantly reduced the F/G ratio compared with the control group ($P<0.05$), while values for remaining groups were intermediate. During Stage II, no significant differences were observed among groups in ADG and F/G ($P>0.05$); however, compared with the control group, 2% plasma protein powder supplementation (Group 1) significantly increased ADFI ($P<0.05$). Additionally, diarrhea incidence in Groups 1, 2, and 4 was significantly lower than that in the control group and Group 3 ($P<0.05$). Over the entire experimental period, ADG and ADFI in Group 1 were significantly higher than those in the control group ($P<0.05$), while values for other groups were intermediate, and no significant differences were detected among groups in F/G ($P>0.05$).

As shown in Table 4 , no significant differences in diarrhea incidence were observed among groups during Stage I ($P>0.05$). During Stage II, the control group and the yeast hydrolysate combined with multi-enzyme preparations group showed significantly higher diarrhea incidence compared with the other groups ($P<0.05$). Over the entire experimental period, no significant differences in diarrhea incidence were detected among groups ($P>0.05$).

2.2 Effects of Combined Application of Yeast Hydrolysate and Multi-Enzyme Preparations or Probiotics on Serum Biochemical Indices of Nursery Piglets

As shown in Table 5 , compared with the yeast hydrolysate group (Group 2), the combination of yeast hydrolysate and multi-enzyme preparations significantly reduced serum triglyceride content ($P<0.05$), while no significant differences were observed among groups for other serum biochemical indices ($P>0.05$).

3.1 Effects of Yeast Hydrolysate on Growth Performance of Nursery Piglets

For early-weaned piglets, supplementation with 3% yeast hydrolysate showed no significant differences in ADG and ADFI compared with 3% plasma protein powder[7], which is consistent with the results of this experiment in nursery piglets. Previous studies have reported that the yeast hydrolysate group exhibited significantly higher ADG and ADFI than the plasma protein powder group, possibly due to higher and different supplementation levels during Stages I and II (5.0% and 2.5%)[8], suggesting that the supplementation ratio of yeast hydrolysate could be adjusted according to different physiological stages of the animals. In this experiment, 0.5% yeast hydrolysate improved the growth performance of nursery piglets by increasing ADFI and ADG, with no significant differences compared with the 2% plasma protein powder group, demonstrating that yeast hydrolysate has the potential to replace plasma protein powder in nursery pig diets.

Yeast hydrolysate contains eight essential amino acids, with its main limiting amino acid ratio of Lys:Met:Thr:Trp = 100:21:19:64, which is close to the recommendation of NRC (1998)[9], and has high effective energy value, providing more energy[10]. Aspartic acid and glutamic acid in yeast hydrolysate are umami amino acids that can enhance the palatability of yeast autolysate, which is one reason for the significantly increased ADFI observed in this experiment.

The nucleotide content in yeast hydrolysate is approximately 8%, which plays a role in promoting small intestinal growth and development and improving intestinal microbial flora. Previous studies have found that dietary supplementation with 0.1% yeast hydrolysate nucleotides during the suckling period can significantly improve weaning stress adaptation and growth performance in piglets[11] and dramatically reduce diarrhea incidence[3], which is basically consistent with the results of this experiment. After weaning, the morphological parameters of the jejunum are significantly affected, while nucleotides can regulate changes in ileal microecology and maintain a stable microflora[12]. Supplementation with 0.05% nucleotides significantly increased villus height and crypt depth and decreased the villus height-to-crypt depth ratio. Additionally, it significantly increased mitotic mucosal cells (M), decreased apoptotic mucosal cells (A), and reduced the A/M index, promoting intestinal development and exerting positive effects on the morphological characteristics of ileal mucosa[13]. The increased small intestinal absorption area facilitates nutrient absorption, which is presumed to be one reason for the significantly increased ADG observed in this experiment.

3.2 Effects of Combined Yeast Hydrolysate and Multi-Enzyme Preparations on Growth Performance of Nursery Piglets

The digestive system of weaned piglets is not fully developed, making them susceptible to weaning stress that affects subsequent growth performance during the nursery period. Supplementation with exogenous protease can compensate for insufficient endogenous enzyme secretion. A combination of cellulase, galactanase, mannanase, and pectinase significantly improved ADG, reduced F/G, dramatically increased total intestinal digestibility, and significantly reduced phosphorus excretion in piglets[14], demonstrating excellent performance in terms of cost-effectiveness and environmental friendliness[15]. Studies have shown that enzyme preparations can reduce viscosity caused by non-starch polysaccharides, increase utilization of amino acids, energy, and phosphorus[16], significantly reduce bacterial metabolites in the stomach[17], and decrease diarrhea incidence[18]. In this experiment, however, the combination of yeast hydrolysate and multi-enzyme preparations increased the diarrhea incidence of nursery piglets during Stage II compared with other groups, possibly due to inadequate exercise of the piglets' own digestive enzyme secretion capacity and excessive fat accumulation leading to diarrhea, which would consequently result in reduced serum triglyceride content.

3.3 Effects of Combined Yeast Hydrolysate and Probiotics on Growth Performance of Nursery Piglets

Supplementation with probiotics in piglet diets can increase ADG, reduce F/G, and decrease diarrhea incidence[19], with no significant differences in growth performance compared with antibiotic groups, suggesting that probiotics can serve as antibiotic alternatives[20]. Reportedly, compound probiotics can effectively reduce serum urea nitrogen concentration, increase nitrogen deposition in the body, and significantly reduce F/G in weaned piglets[21]. Additionally, acid-producing bacteria such as *Bifidobacterium* can lower intestinal pH, destroy the living environment of pathogenic bacteria, reduce *Escherichia coli* counts in feces[22], improve intestinal morphological structure[23], significantly reduce diarrhea incidence, and effectively alleviate stress-induced immune suppression in piglets[24-25]. Moreover, the application of liquid full-fermentation technology has been shown to be superior to solid substrate fermentation in improving pig growth performance, beneficial intestinal microflora, and reducing harmful intestinal microflora[26]. However, increased supplementation levels may reduce growth performance[6], possibly by affecting the balance of intestinal flora. Yeast mannan-oligosaccharides cannot be digested by monogastric animals and tend to accumulate in the colon, while probiotics colonizing the intestine (*Bifidobacterium*, *Lactobacillus*) possess enzymes that can degrade mannan-oligosaccharides and utilize them as an energy source to promote the growth of beneficial bacteria. In this experiment, the combined application of yeast hydrolysate and probiotics showed no significant difference in diarrhea incidence

compared with yeast hydrolysate alone, possibly because yeast hydrolysate itself possesses good intestinal flora regulatory effects.

Conclusions

Dietary supplementation with 2% plasma protein powder or 0.5% yeast hydrolysate can improve growth performance of nursery piglets by increasing ADG and ADFI, and yeast hydrolysate has the potential to replace plasma protein powder.

The combined application of 0.5% yeast hydrolysate with 0.03% probiotics or 0.02% multi-enzyme preparations did not produce synergistic effects on growth performance of nursery piglets.

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