Effects of Three Proteases on Growth Performance, Nutrient Apparent Digestibility, and Serum Biochemical Parameters in Suhuai Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of three proteases on growth performance, nutrient apparent digestibility, and serum biochemical indices in Suhuai weaned piglets. A single-factor randomized block design was adopted. One hundred twenty healthy Suhuai weaned piglets with an average body weight of (9.24±0.84) kg were selected and randomly divided into 4 groups according to the principle of similar body weight and sex. Each group had 6 replicates with 5 piglets per replicate. Piglets in the control group were fed a basal diet without any protease supplementation, while those in the experimental groups were fed the basal diet supplemented with 0.2 g/kg acid protease (Group), 0.2 g/kg neutral protease (Group), and 0.1 g/kg alkaline protease (Group), respectively. The experiment consisted of a 5-day pre-trial period and a 45-day formal trial period. The results showed: 1) Compared with the control group, Group had significantly higher final body weight, average daily gain (ADG), and average daily feed intake (ADFI) ($P<0.05$), while the feed-to-gain ratio (F/G) was reduced but the difference was not significant ($P>0.05$). Group had significantly higher ADG than the control group ($P<0.05$), and its F/G was significantly lower than that of the control group ($P<0.05$). 2) On days 31–45 of the experiment, the diarrhea rate and fecal index of the experimental groups were significantly lower than those of the control group ($P<0.05$), with no significant differences among the experimental groups ($P>0.05$). Group had the lowest diarrhea rate and fecal index. 3) Compared with the control group, the apparent digestibility of dry matter (DM) and organic matter (OM) in the experimental groups were both significantly improved ($P<0.05$). The apparent digestibility of DM and OM in Groups and was significantly higher than that in Group ($P<0.05$). The apparent digestibility of crude protein (CP) in Group was significantly higher than that in the control group, Group , and

Group (P<0.05). 4) Compared with the control group, the serum urea nitrogen content in Group was significantly reduced (P<0.05), with no significant differences among the experimental groups (P>0.05). It can be concluded that acid protease was more effective in improving growth performance and nutrient apparent digestibility in Suhuai weaned piglets, while neutral protease and alkaline protease were more effective in reducing nutritional diarrhea.

Full Text

Effects of Three Proteases on Growth Performance, Nutrient Apparent Digestibility, and Serum Biochemical Indices of Suhuai Weaned Piglets

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Abstract

This experiment investigated the effects of three proteases on growth performance, nutrient apparent digestibility, and serum biochemical indices in Suhuai weaned piglets. A single-factor randomized block design was employed with 120 healthy Suhuai weaned piglets (average body weight: (9.24±0.84) kg) allocated to four groups (six replicates per group, five pigs per replicate). The control group received a basal diet without protease supplementation, while experimental groups received the basal diet supplemented with 0.2 g/kg acid protease (Group I), 0.2 g/kg neutral protease (Group II), or 0.1 g/kg alkaline protease (Group III). The trial consisted of a 5-day adaptation period followed by a 45-day experimental period. The results showed: (1) Compared with the control, Group I exhibited significantly increased final body weight, average daily gain (ADG), and average daily feed intake (ADFI) (P<0.05), with a numerically lower feed-to-gain ratio (F/G) (P>0.05). Group III showed significantly higher ADG (P<0.05) and significantly lower F/G (P<0.05) than the control. (2) During days 31–45, all protease-treated groups had significantly lower diarrhea rates and fecal indices than the control (P<0.05), with no significant differences among experimental groups (P>0.05); Group III demonstrated the lowest values. (3) All experimental groups showed significantly higher dry matter (DM) and organic matter (OM) apparent digestibility than the control (P<0.05), with Groups I and III significantly exceeding Group II (P<0.05). Group I' s crude protein (CP) apparent digestibility was significantly higher than that of the control, Group II, and Group III (P<0.05). (4) Serum urea nitrogen content in Group I was significantly lower than in the control (P<0.05), with no significant differences among experimental groups (P>0.05). These findings indicate that acid protease is most effective for improving growth performance and nutrient

digestibility, while neutral and alkaline proteases are more effective for reducing nutritional diarrhea in Suhuai weaned piglets.

Keywords: protease; weaned piglets; growth performance; nutrient apparent digestibility; serum biochemical indices

Introduction

Plant protein sources such as soybean meal are increasingly used in feed formulations due to their low cost, ready availability, and convenient storage and transportation compared with animal proteins. However, plant proteins contain numerous anti-nutritional factors, including trypsin inhibitors and antigenic proteins, which readily cause nutritional diarrhea in animals, particularly young ones. Exogenous proteases can supplement insufficient endogenous protease secretion, degrade soybean anti-nutritional factors [1], prevent nutritional diarrhea, and improve nutrient digestibility, making them a focus of research in feed applications [2]. Previous studies have shown that protease supplementation can increase daily gain, reduce feed-to-gain ratio, and improve nutrient apparent digestibility in weaned piglets [3]. However, most research has focused on compound enzymes, with no reports comparing the effects of single proteases. This study compared the effects of acid, neutral, and alkaline proteases on growth performance, nutrient apparent digestibility, and serum biochemical indices in Suhuai weaned piglets, with particular emphasis on their efficacy in improving nutritional diarrhea, to provide scientific guidance for rational protease use in feed formulations and healthy piglet production.

Materials and Methods

1.1 Experimental Materials

Acid, neutral, and alkaline proteases were purchased from Shandong Longkote Enzyme Preparation Co., Ltd. Acid protease (from *Aspergillus niger*) had an activity of 100,000 U/g, effective pH range of 2.0–6.0, and optimal pH of 2.5–3.5. Neutral protease (from *Bacillus subtilis*) had an activity of 100,000 U/g, effective pH range of 5.5–8.5, and optimal pH of 6.8–7.0. Alkaline protease (from *Bacillus licheniformis*) had an activity of 200,000 U/g, effective pH range of 6.0–11.0, and optimal pH of 9.5–10.5.

1.2 Experimental Diets and Nutrient Levels

The basal diet was formulated according to NRC (1998) recommendations and nutritional characteristics of Suhuai pigs, prepared as mechanically mixed powder. Basal diet composition and nutrient levels are shown in Table 1 .

1.3 Experimental Animals and Management

A single-factor randomized block design was used. One hundred twenty healthy Suhuai weaned piglets with average body weight of (9.24±0.84) kg were ran-

domly allocated to four groups (six replicates per group, five pigs per replicate) based on similar body weight and gender. The control group received the basal diet, while Groups I, II, and III received the basal diet supplemented with 0.2, 0.2, and 0.1 g/kg of acid protease, neutral protease, and alkaline protease, respectively. The trial included a 5-day adaptation period and a 45-day experimental period. Pigs were housed in a closed facility with partially slatted floors and managed conventionally (fed three times daily at 07:00, 14:00, and 17:00), with regular disinfection, immunization, and pest control. Pigs had ad libitum access to feed and water.

1.4 Measurements and Methods

1.4.1 Growth Performance Pigs were weighed at 08:00 on day 1 and the final day of the experimental period to determine initial and final body weights and calculate average daily gain (ADG). Weekly feed allocation and residual feed were recorded to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated from ADG and ADFI.

1.4.2 Serum Biochemical Indices On the final day, blood samples (10 mL) were collected from the jugular vein of two randomly selected piglets per replicate (one male and one female) before morning feeding. Samples were centrifuged at 3,000 r/min for 20 minutes, and serum was stored at -20°C in 1.5 mL EP tubes for analysis. Serum biochemical indices were measured using the following methods: total protein (TP) by biuret endpoint method; albumin (ALB) by bromocresol green colorimetry; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by spectrophotometry; triglycerides (TG) by enzymatic methods; and urea nitrogen (UN) by urease method. All assay kits were purchased from Beijing Jinhai Keyu Biotechnology Development Co., Ltd.

1.4.3 Diarrhea Rate and Fecal Index Daily diarrhea observations and fecal scoring were recorded for each replicate throughout the trial. The scoring system was: 0 for normal feces (formed pellets), 1 for soft but formed feces (mild diarrhea), 2 for thick, unformed feces without water separation (moderate diarrhea), and 3 for liquid, unformed feces with water separation (severe diarrhea). Antibiotics or antidiarrheal drugs were administered when fecal scores 2. Diarrhea rate and fecal index were calculated at the end of the trial using the following formulas:

Diarrhea rate (%) = 100 × (number of diarrheic pigs) / (total number of pigs in replicate)
Fecal index = sum of fecal scores / total number of pigs

1.4.4 Nutrient Apparent Digestibility A 7-day digestion trial was conducted during the final week, comprising a 3-day adaptation period and a 4-day collection period. One pig per replicate was selected for total fecal collection. Daily feed intake and total fecal output were recorded. Daily fecal samples

(100 g) were mixed with 10 mL of 10% sulfuric acid, frozen, and subsequently pooled across the 4-day collection period for analysis. DM, OM, CP, and ether extract (EE) contents were determined in diets and feces. Nutrient apparent digestibility was calculated as:

Nutrient apparent digestibility (%) = 100 × [nutrient intake (g) -fecal nutrient content (g)] / nutrient intake (g)

1.5 Data Processing

Data were initially processed using Excel 2003 and analyzed by one-way ANOVA using SPSS 22.0. Duncan' s multiple range test was applied for significant differences. Results are expressed as means ± standard deviation, with $P<0.05$ considered statistically significant.

Results

Growth Performance

As shown in Table 2 , Group I (acid protease) had significantly higher ADG and ADFI than the control ($P<0.05$), with a numerically lower F/G ($P>0.05$). Group III (alkaline protease) showed significantly higher ADG ($P<0.05$) and significantly lower F/G ($P<0.05$) compared with the control. Group II (neutral protease) had numerically higher ADG and lower F/G than the control, but differences were not significant ($P>0.05$).

Nutrient Apparent Digestibility

Table 3 shows that all experimental groups had significantly higher DM and OM apparent digestibility than the control ($P<0.05$), with Groups I and III significantly exceeding Group II ($P<0.05$). Group I' s CP apparent digestibility was significantly higher than that of the control, Group II, and Group III ($P<0.05$).

Diarrhea Status

All experimental groups had lower diarrhea rates and fecal indices than the control (Table 4). During days 31-45, all protease-treated groups showed significantly lower diarrhea rates and fecal indices than the control ($P<0.05$), with Group III demonstrating the lowest values.

Serum Biochemical Indices

As shown in Table 5 , Group II had significantly higher serum AST activity than Groups I, III, and the control ($P<0.05$). Serum UN content was lower in all experimental groups than in the control, with Group I showing a significant difference ($P<0.05$).

Discussion

Young animals have underdeveloped gastrointestinal tracts, and weaning causes a substantial reduction in digestive enzyme activities such as pepsin [4], making exogenous protease supplementation crucial. Han et al. [5] reported that dietary supplementation with compound enzymes containing acid protease enhanced intestinal digestive enzyme activities and improved growth performance in nursery pigs. In this study, all experimental groups outperformed the control, consistent with Zuo et al. [6]. Specifically, Group I showed significantly higher final weight, ADG, and ADFI, with a 4.78% reduction in F/G. Group II achieved 5.81%, 8.80%, and 4.27% improvements in final weight, ADG, and ADFI, respectively, with a 4.07% lower F/G. Group III demonstrated significantly higher ADG, with 3.63% and 2.56% improvements in final weight and ADFI, and a significantly lower F/G. Notably, Group I' s final weight was 2.63% and 4.80% higher than Groups II and III, respectively, while ADG was 4.35% and 1.94% higher, and ADFI was 3.28% and 5.00% higher. These results suggest superior efficacy of acid protease for growth performance compared with neutral and alkaline proteases, contrasting with Lu et al. [7] who reported better results for neutral protease. This discrepancy may be attributed to the use of uncoated protease powders in our study. Only acid protease could withstand gastric acidity and function preferentially, enhancing initial CP digestion in the stomach and promoting nutrient absorption in the small intestine. Neutral and alkaline proteases were partially degraded in the acidic gastric environment, reducing their efficacy. Additionally, the enzyme activity levels in our study were an order of magnitude higher than those used by Lu et al. [7], substantially compensating for pepsin deficiency and enhancing gastric digestion. Under ad libitum feeding conditions, piglets' immature digestive systems cannot fully process ingested nutrients, and acid protease can augment gastric digestion, reduce small intestinal burden, and improve growth performance.

Li et al. [8] demonstrated that compound enzyme supplementation containing protease improved CP digestibility in weaned piglets by up to 1.52%, identifying protease as the essential component in enzyme formulations for piglets. In our study, Group I' s CP apparent digestibility increased by 5.32% compared with the control, exceeding Groups II and III by 4.99% and 3.72%, respectively. DM apparent digestibility in Groups I, II, and III increased by 6.14%, 3.22%, and 5.79% over the control, with Groups I and III significantly higher than Group II. OM apparent digestibility improved by 4.81%, 2.22%, and 4.38% in Groups I, II, and III, respectively, with Groups I and III again significantly exceeding Group II. These data indicate that Group I achieved the highest DM, OM, and CP apparent digestibility. The improved CP digestibility across all experimental groups aligns with Yu et al. [9]. Research shows that protease supplementation can enhance CP and amino acid digestibility in the ileum [10], and Zuo et al. [6] similarly reported improved nutrient digestibility. Exogenous proteases may hydrolyze proteins that are resistant to endogenous proteases into peptides and amino acids, thereby improving CP digestibility. While sin-

gle protease preparations are rarely used in livestock production, they enable better comparison of individual enzyme effects and allow investigation of optimal supplementation levels for different growth stages, providing more detailed analysis of digestive metabolism characteristics. Finishing pigs have more developed digestive function than piglets and can digest nutrients more efficiently [11], which may explain why proteases are more effective in piglets.

Weaning stress disrupts the digestive system of piglets, and insufficient digestive enzyme secretion further impairs digestive capacity and health status [12]. Enzyme supplementation can compensate for endogenous enzyme deficiency, aid intestinal nutrient digestion, reduce allergic reactions, and decrease diarrhea rates [13]. Guo et al. [14] recorded a 65.47% reduction in diarrhea rate with acid protease supplementation. In our study, all experimental groups had lower diarrhea rates and fecal indices than the control, with the most pronounced differences observed during days 31–45. Specifically, Groups I, II, and III showed diarrhea rate reductions of 70.44%, 76.67%, and 77.78%, respectively, and fecal index reductions of 56.00%, 66.73%, and 75.82% compared with the control. Group II' s diarrhea rate and fecal index were 21.05% and 24.81% lower than Group I, while Group III' s values were 4.76% and 27.32% lower than Group II. These results suggest that neutral and alkaline proteases are more effective than acid protease for diarrhea prevention, likely because they function more effectively in the intestinal environment. High-protein diets for weaned piglets can cause digestive challenges due to immature digestive and immune systems and unestablished gut microbiota. Undigested proteins reaching the hindgut are degraded by bacteria into harmful amines, causing nutritional diarrhea [15]. Additionally, immune responses to dietary antigens can damage intestinal morphology, impair immune function, and trigger local allergic reactions that exacerbate diarrhea [16]. Neutral protease has an optimal pH of 6.8–7.0, which is well-suited to the small intestinal environment [17]. It supplements insufficient trypsin secretion, promotes protein breakdown, reduces antigen-induced immune stress, and effectively decreases nutritional diarrhea. Soybean by-products contain high residual levels of anti-nutritional factors; while extrusion removes heat-labile factors, it cannot eliminate heat-stable antigenic proteins [18-19], which remain a major cause of piglet diarrhea. Studies show that alkaline protease can improve solubility of soybean proteins and rapidly degrade most antigenic proteins into peptides below 21 kDa, substantially improving soybean meal safety [20]. Additionally, neutral protease secreted by *Bacillus subtilis* can degrade soybean proteins and improve CP digestibility, representing one mechanism of its probiotic effects [21]. Although alkaline protease cannot achieve optimal activity in the intestinal environment, the mildly alkaline conditions remain within its functional range. Alkaline protease effectively degrades anti-nutritional factors such as antigenic proteins [1], converting large, resistant proteins into small peptides that enter tissues and reduce intestinal immune reactions, thereby preventing nutritional diarrhea.

Li et al. [22] found that dietary keratinase supplementation significantly reduced serum ALT activity in piglets. ALT and AST are primarily located in cyto-

plasm and are abundant in heart and liver tissues; their activities reflect liver structural and functional integrity [23]. Liver damage releases large amounts of transaminases into the bloodstream, elevating serum levels. In our study, Group II showed significantly higher serum AST activity than Groups I, III, and the control, which is inconsistent with previous reports and requires further investigation. Group I had significantly lower serum UN content than the control. Blood urea, synthesized via the ornithine cycle, is the final product of protein catabolism and reflects protein metabolism. In monogastric animals, serum UN content is inversely related to dietary nitrogen utilization and can assess protein biological value; lower UN indicates higher protein utilization efficiency [24]. Groups I, II, and III showed 1.69%, 2.78%, and 2.42% higher serum TP content than the control, though differences were not significant. TP functions in nutrient transport, osmotic pressure maintenance, and humoral immunity; increased TP content can enhance nutrient transport and maintain homeostasis.

Conclusion

Dietary supplementation with acid, neutral, and alkaline proteases improved growth performance, nutrient digestibility, and reduced diarrhea rates in Suhuai weaned piglets. Group I achieved higher final weight, ADG, ADFI, and apparent digestibility of DM, OM, and CP compared with Groups II and III, while Groups II and III showed lower diarrhea rates and fecal indices than Group I. These results demonstrate that acid protease is most effective for improving growth performance and nutrient digestibility, whereas neutral and alkaline proteases are more effective for reducing nutritional diarrhea.

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