

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

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Abstract

This experiment was conducted to investigate the effects of three proteases on growth performance, apparent nutrient digestibility, and serum biochemical indices in Suhuai weaned piglets. A single-factor randomized block design was adopted. A total of 120 healthy Suhuai weaned piglets with an average body weight of (9.24 ± 0.84) kg were selected and randomly allocated to 4 groups based on the principle of similar body weight and sex, with 6 replicates per group and 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 I), 0.2 g/kg neutral protease (Group II), and 0.1 g/kg alkaline protease (Group III), respectively. The experiment consisted of a 5-day pre-trial period followed by a 45-day formal trial period. The results showed: 1) Compared with the control group, Group I exhibited significantly higher final body weight, average daily gain (ADG), and average daily feed intake (ADFI) ($P < 0.05$), and a lower feed-to-gain ratio (F/G), although the difference in F/G was not significant ($P > 0.05$). The ADG of Group III was significantly higher than that of the control group ($P < 0.05$), and the F/G was significantly lower than that of the control group ($P < 0.05$). 2) During 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 ($P > 0.05$), and Group III 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 was significantly improved ($P < 0.05$). The apparent digestibility of DM and OM in Groups I and III was significantly higher than that in Group II ($P < 0.05$). The apparent digestibility of crude protein (CP) in Group I was significantly

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

Full Text

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

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Abstract

This experiment was conducted 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 employed, allocating 120 healthy Suhuai weaned piglets with an average body weight of (9.24 ± 0.84) kg into four groups. The groups consisted of six replicates each with five pigs, blocked by similar body weight and sex. The control group received a basal diet without any 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 included a 5-day adaptation period followed by a 45-day experimental period. The results demonstrated: (1) Compared with the control, Group I showed significantly increased final weight, average daily gain (ADG), and average daily feed intake (ADFI) ($P < 0.05$), while feed-to-gain ratio (F/G) decreased without reaching significance ($P > 0.05$). Group III exhibited 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 achieved the lowest values. (3) All experimental groups showed significantly higher apparent digestibility of dry matter (DM) and organic matter (OM) compared with the control ($P < 0.05$), with Groups I and III significantly exceeding Group II ($P < 0.05$). Group I demonstrated significantly higher crude protein (CP) apparent digestibility than 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 ($P < 0.05$), though no significant differences existed among experimental groups ($P > 0.05$). These findings indicate that acid protease most effectively improves growth performance and nutrient apparent digestibility in Suhuai weaned piglets, while neutral and alkaline proteases are more effective in reducing nutritional diarrhea.

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

Introduction

Plant-based protein sources such as soybean meal have become increasingly prevalent in feed formulations due to their low cost, ready availability, and convenient storage and transportation compared with animal proteins. However, these ingredients contain numerous anti-nutritional factors, including trypsin inhibitors and antigenic proteins, which readily induce nutritional diarrhea in animals, particularly young ones. Exogenous proteases not only compensate for insufficient endogenous protease secretion and degrade soybean anti-nutritional factors to prevent nutritional diarrhea, but also enhance nutrient digestibility, making them a focus of intensive research in feed applications. Previous studies have demonstrated that protease supplementation can increase daily gain, reduce feed-to-gain ratio, and improve nutrient apparent digestibility in weaned piglets. However, most existing research has focused on compound enzymes, with no comparative reports on the efficacy of individual proteases. This study aims to compare 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 ameliorating nutritional diarrhea, thereby providing scientific guidance for rational protease application in feed formulations and healthy piglet production.

1. Materials and Methods

1.1 Experimental Materials Acid, neutral, and alkaline proteases were purchased from Shandong Longkete Enzyme Preparation Co., Ltd. The acid protease, derived from *Aspergillus niger*, had an activity of 100,000 U/g with an effective pH range of 2.0-6.0 and optimal pH of 2.5-3.5. The neutral protease, derived from *Bacillus subtilis*, exhibited 100,000 U/g activity, effective pH 5.5-8.5, and optimal pH 6.8-7.0. The alkaline protease, derived from *Bacillus licheniformis*, possessed 200,000 U/g activity, effective pH 6.0-11.0, and optimal pH 9.5-10.5.

1.2 Experimental Diets and Nutrient Levels The basal diet formulation was designed according to NRC (1998) recommendations and tailored to the nutritional characteristics of Suhuai pigs. Experimental diets were mechanically

mixed as powder. The composition and nutrient levels of the basal diet are presented in .

1.3 Experimental Animals and Management A single-factor randomized block design was employed. One hundred twenty healthy Suhuai weaned piglets with an average body weight of (9.24 ± 0.84) kg were randomly allocated into four groups (six replicates per group, five pigs per replicate) based on similar body weight and sex distribution. The control group received the basal diet, while Groups I, II, and III received the basal diet supplemented with 0.2 g/kg acid protease, 0.2 g/kg neutral protease, and 0.1 g/kg alkaline protease, respectively. The trial consisted of a 5-day adaptation period followed by a 45-day experimental period. Pigs were housed in enclosed facilities with semi-slatted floors and managed conventionally, receiving feed three times daily at 07:00, 14:00, and 17:00. Regular disinfection, immunization, and pest control were implemented, with 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, from which average daily gain (ADG) was calculated. Weekly feed allocation and residual amounts were recorded precisely to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was derived from ADG and ADFI.

1.4.2 Serum Biochemical Indices On the final experimental 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 immediately centrifuged at 3,000 rpm for 20 minutes, and serum was stored in 1.5 mL EP tubes at -20°C until analysis. Serum biochemical parameters were determined 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 assay; 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 per replicate throughout the trial using the following scale: 0 for normal formed feces, 1 for soft but formed feces (mild diarrhea), 2 for thick unformed feces without water separation (moderate), and 3 for liquid feces with water separation (severe). Antibiotics or antidiarrheal treatments were administered when fecal scores ≥ 2 . Diarrhea rate and fecal index were calculated at trial completion using these formulas:

- Diarrhea rate (%) = $100 \times (\text{number of diarrheic pigs}) / (\text{total pigs per replicate})$

- Fecal index = sum of fecal scores / total pigs per replicate

1.4.4 Nutrient Apparent Digestibility A digestion trial was conducted during the final week, comprising a 3-day adaptation and 4-day collection period. One pig per replicate was selected for total fecal collection, with daily feed intake and total fecal output 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. Diets and feces were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE). Nutrient apparent digestibility was calculated as:

Nutrient apparent digestibility (%) = $100 \times [\text{nutrient intake (g)} - \text{fecal nutrient excretion (g)}] / \text{nutrient intake (g)}$

1.5 Data Processing Experimental data were initially processed using Excel 2003, followed by one-way ANOVA using SPSS 22.0. Duncan's multiple range test was applied when significant differences were detected. Results are expressed as means \pm standard deviation, with $P < 0.05$ considered statistically significant.

2. Results

2.1 Effects of Three Proteases on Growth Performance of Weaned Piglets As shown in , Group I exhibited significantly higher ADG and ADFI than the control ($P < 0.05$), with F/G decreasing without statistical significance ($P > 0.05$). Group III showed significantly higher ADG ($P < 0.05$) and significantly lower F/G ($P < 0.05$) compared with the control. Group II demonstrated higher ADG and lower F/G than the control, though differences were not significant ($P > 0.05$).

2.2 Effects of Three Proteases on Nutrient Apparent Digestibility of Weaned Piglets reveals 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 showed significantly higher CP apparent digestibility than the control, Group II, and Group III ($P < 0.05$).

2.3 Effects of Three Proteases on Diarrhea in Weaned Piglets All protease-treated groups exhibited lower diarrhea rates and fecal indices than the control. During days 31–45, these differences became statistically significant ($P < 0.05$), with Group III achieving the lowest values ().

2.4 Effects of Three Proteases on Serum Biochemical Indices of Weaned Piglets As presented in , Group II showed 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 only Group I reaching statistical significance ($P < 0.05$).

3. Discussion

3.1 Effects on Growth Performance The gastrointestinal tract of young animals is underdeveloped, and weaning causes a substantial decline in digestive enzyme activities, including gastric protease. Supplementing exogenous protease is therefore crucial. Han et al. reported that dietary complex enzymes containing acid protease enhanced intestinal digestive enzyme activities and improved growth performance in nursery pigs. In this study, all protease groups outperformed the control, consistent with Zuo et al. Group I showed significantly higher final weight, ADG, and ADFI, with F/G decreasing by 4.78% compared with the control. Group II increased final weight, ADG, and ADFI by 5.81%, 8.80%, and 4.27%, respectively, while reducing F/G by 4.07%. Group III achieved significantly higher ADG than the control, with final weight and ADFI increasing by 3.63% and 2.56%, respectively, and F/G decreasing significantly. Notably, Group I exceeded Groups II and III in final weight (by 2.63% and 4.80%), ADG (by 4.35% and 1.94%), and ADFI (by 3.28% and 5.00%), indicating superior efficacy of acid protease for growth performance. This contrasts with Lu et al., who found neutral protease superior to acid protease. The discrepancy likely arises because our proteases were uncoated powders; only acid protease could withstand gastric acidity to function effectively in the stomach, enhancing preliminary CP digestion and promoting subsequent small intestinal absorption. Neutral and alkaline proteases were partially denatured in the acidic stomach environment, reducing their efficacy. Moreover, our protease activity levels were an order of magnitude higher than those in Lu et al., substantially compensating for gastric protease insufficiency and enhancing gastric digestion. Under ad libitum feeding, piglets' immature digestive systems cannot fully process ingested nutrients; acid protease strengthens gastric digestion, reduces small intestinal burden, and thereby improves growth performance.

3.2 Effects on Nutrient Digestibility Li et al. demonstrated that protease-containing complex enzymes could improve CP digestibility in weaned piglets by up to 1.52%, identifying protease as an essential component in enzyme formulations. In this study, Group I increased CP apparent digestibility by 5.32% over the control, and by 4.99% and 3.72% over Groups II and III, respectively. DM apparent digestibility in Groups I, II, and III increased by 6.14%, 3.22%, and 5.79% compared with the control, with Groups I and III significantly exceeding Group II. OM apparent digestibility increased by 4.81%, 2.22%, and 4.38% across the three groups, again with Groups I and III surpassing Group II. These results demonstrate that Group I achieved the highest DM, OM, and CP apparent digestibility. All experimental groups showed improved CP di-

gestibility over the control, consistent with Yu et al. Research indicates that protease supplementation can enhance ileal digestibility of CP and amino acids, as corroborated by Zuo et al. Exogenous proteases may hydrolyze proteins that resist endogenous enzymes into peptides and amino acids, thereby improving CP digestibility. While single protease preparations are rarely used in livestock production, they enable better comparison of individual enzyme efficacy and facilitate investigation of optimal supplementation levels for different growth stages, allowing more detailed analysis of digestive metabolism characteristics. Growing pigs possess more developed digestive function than piglets and can digest nutrients more efficiently, making protease supplementation more effective in piglets.

3.3 Effects on Diarrhea Weaning stress disrupts the digestive system of piglets, and insufficient digestive enzyme secretion further impairs digestive capacity, compromising health status. Enzyme supplementation can compensate for endogenous enzyme deficiency, aid intestinal nutrient digestion, reduce allergic reactions, and decrease diarrhea incidence. Guo et al. reported that acid protease reduced diarrhea rate by 65.47% in piglets. In this study, all experimental groups exhibited lower diarrhea rates and fecal indices than the control, with the most pronounced differences occurring during days 31–45. Specifically, Groups I, II, and III reduced diarrhea rates by 70.44%, 76.67%, and 77.78%, respectively, and decreased fecal indices by 56.00%, 66.73%, and 75.82% compared with the control. Group II reduced diarrhea rate and fecal index by 21.05% and 24.81% compared with Group I, while Group III decreased these parameters by 4.76% and 27.32% relative to Group II. These findings suggest that neutral and alkaline proteases are more effective than acid protease in preventing diarrhea, likely because they function more prominently in the intestine. High-protein diets for weaned piglets, combined with immature digestive and immune systems and unstable intestinal microbiota, create digestive barriers to dietary CP. Undigested proteins reaching the hindgut are degraded by bacteria into harmful amines, causing nutritional diarrhea. Additionally, immune responses to dietary antigens damage intestinal morphology and immune function, triggering local allergic reactions that exacerbate diarrhea. Neutral protease, with an optimal pH of 6.8–7.0, is well-suited to the small intestinal environment. It compensates for insufficient trypsin secretion, promotes protein breakdown, reduces antigen-induced immune stress, and effectively decreases nutritional diarrhea. Soybean byproducts contain substantial residual anti-nutritional factors; while extruded soybean eliminates heat-labile factors through high-temperature processing, it cannot remove heat-stable soybean antigen proteins, which remain a major cause of piglet diarrhea. Studies have shown that alkaline protease can improve solubility of soybean protein and rapidly degrade most soybean antigen proteins into peptides below 21 kDa, substantially improving the safety of soybean meal. Additionally, neutral protease secreted by *Bacillus subtilis* can degrade soybean protein and improve CP digestibility, representing one mechanism of its probiotic effects. Although alkaline protease cannot achieve optimal activity in the

intestinal environment, the mildly alkaline conditions still fall within its functional range. Alkaline protease effectively degrades anti-nutritional factors such as antigenic proteins, converting large, indigestible proteins into small peptides that enter tissues, thereby reducing intestinal immune reactions triggered by anti-nutritional factors and preventing nutritional diarrhea.

3.4 Effects on Serum Biochemical Indices Li et al. found that dietary keratinase supplementation significantly reduced serum ALT activity in piglets. ALT and AST are primarily located in cellular fluid, particularly abundant in heart and liver tissues, and their activities reflect hepatic structural and functional integrity. Elevated serum transaminase levels indicate liver damage with enzyme leakage into circulation. In this study, Group II showed significantly higher serum AST activity than the control, Group I, and Group III, a finding inconsistent with previous research and requiring further investigation. Group I exhibited significantly lower serum UN content than the control. Blood urea, synthesized via the ornithine cycle as the final product of protein catabolism, reflects protein metabolism. In monogastric animals, serum UN content inversely correlates with dietary nitrogen utilization and indicates protein biological value—lower UN signifies higher protein utilization efficiency. Groups I, II, and III increased serum TP content by 1.69%, 2.78%, and 2.42% compared with the control, though differences were not significant. TP plays vital roles in nutrient transport, osmotic pressure maintenance, and humoral immunity; increased TP levels enhance nutrient transport and maintain homeostasis.

4. Conclusion

This study demonstrated that 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 exhibited lower diarrhea rates and fecal indices than Group I. These results indicate that acid protease is most effective for enhancing growth performance and nutrient digestibility, whereas neutral and alkaline proteases are superior for reducing nutritional diarrhea.

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