Comparison of the effects of fungal- and bacterial-produced xylanases on growth performance, small intestinal villus morphology, and blood biochemical indices in yellow-feathered broilers: Postprint

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Abstract

The present study was conducted to investigate the effects of two xylanases on growth performance, digestive organ development, small intestinal villus morphology, and blood biochemical indices in yellow-feathered broilers. A total of 540 healthy 1-day-old yellow-feathered broilers were randomly divided into 3 groups with 6 replicates per group and 30 broilers per replicate. The control group was fed a corn-wheat-soybean meal basal diet, while experimental groups A and B were fed the basal diet supplemented with 200 g/t fungal xylanase and 200 g/t bacterial xylanase, respectively. The experimental period lasted 42 days. The results showed that, compared with the control group: 1) The average daily gain of the experimental groups was significantly increased ($P<0.05$), and the feed conversion ratio was decreased to a certain extent, with group A showing a significant decrease ($P<0.05$); 2) Serum glucose content and alkaline phosphatase and creatine kinase activities were significantly increased ($P<0.05$), while serum triglyceride and urea nitrogen contents were significantly decreased ($P<0.05$); 3) The relative weights of the proventriculus and pancreas were significantly decreased ($P<0.05$); 4) The jejunal villus height in groups A and B increased by 14.81% and 11.04% ($P<0.05$), respectively, and the jejunal villus height/crypt depth ratio increased by 16.61% and 12.70% ($P<0.05$), respectively. It can be concluded that supplementation of 200 g/t fungal or bacterial xylanase in the diet of yellow-feathered broilers can improve small intestinal villus development and immune function, enhance growth performance, and the effects did not differ significantly between the two enzymes.

Full Text

Comparison of Effects of Fungal and Bacterial Xylanase on Growth Performance, Small Intestinal Villus Morphology, and Blood Biochemical Indices in Yellow-Feathered Broilers

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Abstract

This study investigated the effects of two types of xylanase on growth performance, digestive organ development, small intestinal villus morphology, and blood biochemical indices in yellow-feathered broilers. A total of 540 one-day-old healthy yellow-feathered broilers were randomly allocated into three groups, each consisting of six replicates of 30 birds. The control group received a corn-wheat-soybean meal basal diet, while experimental groups A and B were fed the basal diet supplemented with 200 g/t of fungal xylanase and bacterial xylanase, respectively. The experiment lasted 42 days. Compared with the control group, the results showed that: 1) average daily gain was significantly increased in both experimental groups ($P<0.05$), and feed-to-gain ratio decreased, with group A showing a significant reduction ($P<0.05$); 2) serum glucose content and activities of alkaline phosphatase and creatine kinase were significantly elevated ($P<0.05$), while serum triglyceride and urea nitrogen contents were significantly decreased ($P<0.05$); 3) the relative weights of proventriculus and pancreas were significantly reduced ($P<0.05$); and 4) jejunal villus height in groups A and B increased by 14.81% and 11.04% ($P<0.05$), respectively, and the villus height-to-crypt depth ratio improved by 16.61% and 12.70% ($P<0.05$). These findings indicate that supplementation with 200 g/t of either fungal or bacterial xylanase can improve small intestinal villus development and immune function, enhance growth performance in yellow-feathered broilers, with no significant differences between the two enzyme sources.

Keywords: xylanase; wheat-based diets; yellow-feathered broilers; small intestinal villus

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Wheat is a high-quality energy feed ingredient commonly used to partially replace corn in poultry diets to reduce feed costs. Although wheat contains higher levels of protein, amino acids, calcium, and available phosphorus than corn, its high arabinoxylan content (approximately 8%) limits its extensive application in livestock and poultry diets. Numerous studies have confirmed that xylanase supplementation in wheat-based diets can eliminate or reduce the anti-nutritional effects of arabinoxylans, decrease intestinal chyme viscosity, improve nutrient

digestion, absorption, and metabolism, regulate blood metabolic hormone levels, and enhance poultry production performance. Xylanases can be classified as fungal or bacterial based on their source, with most xylanases derived from fungi such as *Trichoderma*, *Aspergillus niger*, and *Aspergillus oryzae*. Bacterial xylanases primarily originate from *Pseudomonas fluorescens* subsp. *cellulosa*, *Ruminococcus flavefaciens*, *Cellulomonas fimi*, *Fibrobacter succinogenes*, *Clostridium thermocellum*, and *Clostridium stercorarium*. Fungal and bacterial xylanases differ in their enzyme profiles, modes of action, and characteristics, yet few studies have compared their effects and mechanisms of action. Therefore, this experiment used yellow-feathered broilers fed corn-wheat-soybean meal diets to compare the effects of fungal and bacterial xylanases on growth performance, digestive organ development, small intestinal villus morphology, and blood biochemical indices, providing a basis for xylanase development and application in yellow-feathered broiler feed production.

1.1 Experimental Materials

Fungal xylanase (produced by *Trichoderma reesei*) and bacterial xylanase (produced by genetically modified *Bacillus*), both with an activity of 30,000 IU/g, were provided by Shandong Longda Bio-Engineering Co., Ltd. One enzyme activity unit (IU) was defined as the amount of enzyme required to release 1 mol of reducing sugar per minute under conditions of 37°C and pH 5.5.

1.2 Experimental Design and Diets

1.2.1 Experimental Design A total of 540 one-day-old yellow-feathered broilers were randomly divided into three groups with six replicates each, containing 30 birds per replicate (mixed sex). The control group received a corn-wheat-soybean meal basal diet, experimental group A received the basal diet supplemented with 200 g/t fungal xylanase, and experimental group B received the basal diet supplemented with 200 g/t bacterial xylanase. The experimental period lasted 42 days.

1.2.2 Basal Diet The basal diet was formulated according to the *NY/T 33 −2004 Feeding Standard of Chickens* and NRC (1994) nutrient requirements for broilers. The composition and nutrient levels of the basal diet are presented in Table 1 .

1.2.3 Management Practices Birds were housed in three-tier full-step cage systems with ad libitum access to feed and water. Conventional broiler management practices were followed. Daily observations included recording house temperature and humidity, monitoring flock health status, and promptly removing and recording any dead birds.

1.3 Measurements

1.3.1 Growth Performance During the experimental period, daily feed intake and mortality were recorded per replicate. At 42 days of age, birds were weighed after a 12-hour fast at 08:00, and remaining feed weights were recorded. Feed consumption was calculated per replicate to determine average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.3.2 Digestive Organ Relative Weight and Small Intestinal Villus Morphology **Digestive Organ Relative Weight:** On day 42, one bird per replicate (six per group) was randomly selected, slaughtered, and dissected. The gizzard (after removing contents and cuticle), proventriculus, and pancreas were excised, trimmed of fat, and weighed fresh. Relative weight was calculated as: (organ weight in g) / (live weight in kg).

Small Intestinal Villus Morphology: During slaughter, the jejunum and ileum were removed, and 2-cm mid-sections were cut. These segments were gently rinsed with saline, flattened on filter paper, trimmed, and fixed in 10% formalin solution for over 24 hours. Samples were then processed through washing, dehydration, clearing, paraffin infiltration, embedding, sectioning, and hematoxylin-eosin staining. Villus morphology was observed under an electron microscope, with typical fields photographed and measured using DT2000 universal image analysis software v2.0. Villus height and crypt depth were measured in jejunum and ileum, with six readings averaged per metric, and villus height-to-crypt depth ratio calculated.

1.3.3 Serum Biochemical Indices Blood samples (10–20 mL) were collected via jugular vein puncture, allowed to clot for 30 minutes, then centrifuged at 3,500 rpm for 10 minutes to separate serum, which was stored at -20°C. Serum urea nitrogen was determined by the urease method, glucose by the oxidase method, alkaline phosphatase activity by the enzyme rate method, total protein by the biuret method, triglycerides by the enzymatic method, and creatine kinase activity by the enzyme coupling method. Triiodothyronine, thyroxine, and insulin-like growth factor were measured by electrochemistry, and growth hormone by radioimmunoassay, following kit instructions.

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 19.0 software, and Duncan' s multiple range test was used for pairwise comparisons. Significance was declared at $P<0.05$. Data are expressed as means ± standard deviation (mean±SD).

2.1 Effects of Different Xylanase Sources on Growth Performance

As shown in Table 2 , compared with the control group, both experimental groups A and B exhibited significantly higher average daily gain ($P<0.05$). Feed-

to-gain ratio was significantly reduced in group A ($P<0.05$) and decreased in group B, though not significantly ($P>0.05$). No significant differences were observed between groups A and B for ADFI, ADG, or F/G ($P>0.05$). These results indicate that both xylanases improved broiler growth performance without significant differences between them.

2.2 Effects of Different Xylanase Sources on Digestive Organ Relative Weight

Table 3 shows that compared with the control group, the relative weight of proventriculus decreased by 19.44% ($P<0.05$) and 19.23% ($P<0.05$) in groups A and B, respectively, while pancreatic relative weight decreased by 8.09% ($P<0.05$) and 6.94% ($P<0.05$), respectively. Gizzard relative weight showed no significant difference ($P>0.05$). No significant differences were found between groups A and B for any digestive organ relative weight ($P>0.05$). These results demonstrate that both xylanases reduced digestive organ relative weight, particularly for proventriculus and pancreas.

2.3 Effects of Different Xylanase Sources on Small Intestinal Villus Morphology

Table 4 reveals that compared with the control group, jejunal villus height increased by 14.81% and 11.04% in groups A and B, respectively ($P<0.05$). Although jejunal crypt depth showed a decreasing trend with xylanase supplementation ($P>0.05$), the villus height-to-crypt depth ratio significantly improved by 16.61% and 12.70% ($P<0.05$). Ileal villus height was also significantly increased in both experimental groups ($P<0.05$), though crypt depth remained unaffected ($P>0.05$). The ileal villus height-to-crypt depth ratio was significantly higher in group A than in the control ($P<0.05$), but no significant difference was observed for group B ($P>0.05$). No significant differences were detected between groups A and B for any parameter ($P>0.05$).

Histological examination of jejunal and ileal paraffin sections (Figures 1 [Figure 1: see original paper] and 2 [Figure 2: see original paper]) showed that control group villi were poorly developed, severely damaged, short, irregularly arranged, and exhibited some sloughing. In contrast, villi in both experimental groups were better developed, longer, more regularly shaped, and densely arranged, with group A showing particularly long, clearly outlined, and well-developed villi.

2.4 Effects of Different Xylanase Sources on Serum Biochemical Indices

Table 5 demonstrates that compared with the control group, serum urea nitrogen decreased significantly by 12.19% and 10.98% in groups A and B, respectively ($P<0.05$). Alkaline phosphatase activity increased significantly by 69.40% and 55.19% ($P<0.05$), while triglyceride content decreased significantly by 40.23%

and 36.78% ($P<0.05$). Serum glucose content increased significantly by 25.06% and 15.63% ($P<0.05$). Growth hormone content increased significantly by 9.09% in group A ($P<0.05$) but only showed a non-significant increase in group B ($P>0.05$). Creatine kinase activity increased significantly by 21.90% and 14.41% in groups A and B, respectively ($P<0.05$). No significant differences were observed between groups A and B for any index ($P>0.05$). Serum total protein, insulin-like growth factor, triiodothyronine, and thyroxine contents showed no significant differences among groups ($P>0.05$). These results indicate that both xylanases significantly reduced serum urea nitrogen and triglyceride contents while increasing alkaline phosphatase and creatine kinase activities and glucose content, but had no significant effects on total protein, insulin-like growth factor, triiodothyronine, or thyroxine.

3.1 Effects of Different Xylanase Sources on Broiler Growth Performance

Wheat is limited in feed production due to its high arabinoxylan content, but xylanase can disrupt the integrity of arabinoxylan anti-nutritional factors, thereby reducing feed viscosity, promoting nutrient digestion and absorption, and improving animal production performance. Numerous studies have shown that xylanase supplementation in wheat-based diets improves poultry growth performance. Lü et al. found that xylanase significantly reduced feed-to-gain ratio and average daily feed intake in broilers during both 1–21 and 22–42 day periods, with combined enzymes showing superior effects to single enzymes. Our results demonstrate that both xylanases significantly improved average daily gain and reduced feed-to-gain ratio, with fungal xylanase showing a greater reduction in feed-to-gain ratio than bacterial xylanase, possibly due to differences in enzyme composition, characteristics, and mechanisms of action between sources.

3.2 Effects of Different Xylanase Sources on Digestive Organ Relative Weight

The degree of digestive tract development determines animal growth rate, manifesting both in functional maturation and changes in digestive organ indices. Research indicates that digestive organ relative weight correlates with intestinal chyme viscosity, with higher viscosity leading to greater organ weights. Additionally, non-starch polysaccharide enzymes can reduce intestinal weight in diets containing these compounds. Wang et al. reported that xylanase supplementation in wheat-based diets tended to reduce intestinal weight, decreasing the relative weights of pancreas, liver, small intestine, gizzard, and proventriculus, though differences were not significant. Lü et al. showed that different xylanases tended to reduce proventriculus and pancreas relative weights, with uncoated xylanase at 30,000 U/g showing the greatest reduction. In our study, xylanase did not affect gizzard relative weight but significantly reduced proventriculus and pancreas relative weights. This may be because xylanase accelerated the degradation of non-starch polysaccharides in the stomach, reducing chyme vis-

cosity and improving nutrient metabolism, thereby decreasing the digestive burden and organ weights. Water-soluble arabinoxylan in wheat is considered the primary anti-nutritional component in poultry diets, as it increases intestinal content viscosity, alters microbial flora, reduces enzyme-substrate contact, and impedes digestion, leading to structural and functional changes in digestive organs. To adapt to these changes, intestinal secretory mechanisms are upregulated, causing compensatory hyperplasia of digestive organs. Xylanase supplementation partially hydrolyzes arabinoxylans in wheat-based diets, weakening the endocrine response and potentially reducing digestive organ relative weights.

3.3 Effects of Different Xylanase Sources on Small Intestinal Villus Morphology

The small intestine is the primary site for nutrient absorption in poultry, and its normal structure is essential for adequate digestion and absorption. Villus height, crypt depth, and their ratio are important indicators of small intestinal digestive and absorptive function. Villus development is mainly influenced by the intestinal environment, and chyme viscosity is a critical environmental factor closely related to villus development. Our results show that xylanase significantly increased villus height in both jejunum and ileum and improved the jejunal villus height-to-crypt depth ratio. Histological examination also revealed better villus development in both enzyme-supplemented groups compared with the control. Jiang obtained similar results in yellow-feathered broilers fed wheat-based diets supplemented with fungal and bacterial xylanases, showing increased duodenal villus height, shallower crypt depth, and improved villus height-to-crypt depth ratio without significant differences between enzyme sources. Mathlouthi et al. also confirmed that broilers fed rye-based diets had significantly lower villus height and width than those fed corn-based diets, but xylanase and β-glucanase supplementation significantly improved these parameters and maintained normal intestinal morphology. This may be because xylanase degraded arabinoxylans in wheat-based diets, reducing intestinal chyme viscosity and decreasing harmful microbial proliferation while increasing beneficial microbes, thereby reducing or eliminating damage to intestinal villi. Additionally, xylanase can promote nutrient digestion in chyme, increasing the amount of absorbable nutrients such as small molecular amino acids and oligosaccharides (e.g., xylobiose, xylotriose), which can be directly utilized as nutrients by intestinal mucosa, thus promoting villus growth and development.

3.4 Effects of Different Xylanase Sources on Serum Biochemical Indices

Urea nitrogen content shows a significant negative correlation with protein utilization efficiency, and serum urea nitrogen accurately reflects protein metabolism and dietary amino acid balance in animals, decreasing when protein metabolism is favorable. In our study, xylanase supplementation did not significantly affect serum total protein content, likely due to the dietary

ingredient composition and nutrient levels, further indicating that blood total protein is influenced by diet type and nutrient level. However, both xylanases significantly reduced serum urea nitrogen content, demonstrating effective elimination of arabinoxylan anti-nutritional effects, reduced chyme viscosity, and improved crude protein metabolism.

Alkaline phosphatase is a non-specific membrane-bound enzyme that hydrolyzes various phospholipids and participates in lipid metabolism, with its activity closely related to growth. Serum alkaline phosphatase activity is also associated with osteoblast and liver excretory function, primarily produced and released by osteoblasts, and is closely related to bone tissue growth. It serves as a biochemical indicator of osteoblast activity and bone formation status, as well as calcium and phosphorus metabolism. The significant effect of xylanase on alkaline phosphatase activity in our study suggests that xylanase supplementation can promote lipid, calcium, and phosphorus metabolism, thereby enhancing growth.

Creatine kinase is an organ-specific enzyme primarily found in skeletal muscle, cardiac muscle, and brain tissue. It participates in energy metabolism by reversibly catalyzing the reaction between creatine and ATP to generate phosphocreatine and ADP. At neutral pH, creatine kinase primarily promotes the reverse reaction, favoring ATP generation to ensure cellular energy supply. Our finding that both xylanases significantly increased creatine kinase activity indicates that xylanase supplementation can improve energy metabolism and enhance broiler growth performance.

Triglycerides are the primary form of energy storage in the body, and their content is a major indicator of lipid metabolism. Our results show that both xylanases significantly reduced serum triglyceride content, indicating that xylanase supplementation can improve lipid metabolism in broilers.

Serum glucose content reflects the level of glucose absorbed from the intestine into the blood and is directly related to dietary carbohydrate digestibility. Zhang et al. found that supplementing broiler diets with 1,000 U/kg xylanase significantly increased blood glucose content, and Vit et al. confirmed that 0.2% xylanase supplementation in wheat-based diets significantly increased blood glucose in geese. Our results show that both xylanases significantly increased serum glucose content in broilers, suggesting that xylanase can improve dietary starch digestibility, with fungal xylanase showing a greater increase than bacterial xylanase.

Triiodothyronine and thyroxine are hormones that widely participate in regulating metabolism, including serum glucose, lipid, and protein metabolism. Growth hormone redistributes absorbed nutrients among tissues, promoting bone, cartilage, and tissue growth. Insulin-like growth factor is synthesized primarily in the liver and acts as a growth-promoting factor. In our study, both xylanases tended to promote growth hormone secretion but showed no significant effects on triiodothyronine, thyroxine, or insulin-like growth factor. This aligns

with Wang et al., who found no significant differences in serum triiodothyronine and thyroxine in 28-day-old broilers fed wheat-based diets with xylanase supplementation. However, Han reported that crude enzyme supplementation in barley-based diets affected poultry blood metabolic hormones including thyroid hormones, insulin, and growth hormone. Kelley also reported increased levels of triiodothyronine and growth hormone after non-starch polysaccharide enzyme supplementation. These discrepancies may be related to differences in enzyme type, composition, and activity used in various studies.

Conclusion

Appropriate supplementation of either fungal or bacterial xylanase in corn-wheat-soybean meal diets can improve small intestinal villus morphology and immune function, enhance growth performance in yellow-feathered broilers, with no significant differences between the two enzyme sources.

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