Effects of Xylanases from Different Sources and Their Combinations on Intestinal Mucosal Morphology, Disaccharidase Activity, and Its Gene Expression in Broiler Chickens (Postprint)

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Abstract

This experiment aimed to investigate the effects of xylanase from different sources and their combinations on intestinal mucosal morphology, disaccharidase activity, and gene expression in yellow-feathered broilers. A total of 1,560 one-day-old yellow-feathered male broilers were selected and randomly divided into 4 groups (6 replicates per group, 65 birds per replicate): the control group (Group) was fed a basal diet low in energy and protein, while the experimental groups (Groups , , and) were fed the basal diet supplemented with 4,000 U/kg of single xylanase 1# from Aspergillus niger, single xylanase 2# from Trichoderma koningii, and combined xylanase (xylanase 1#: xylanase 2# = 1:1), respectively, for a 63-day experimental period. From each replicate, 2 broilers were selected at 21, 42, and 63 days of age to collect intestinal tissues for determination of intestinal mucosal morphology, disaccharidase activity, and mRNA expression abundance of disaccharidase genes. The results showed: 1) At 21 days of age, the crypt depth in the jejunum of Group was significantly lower than that of Group (P<0.05); at 42 days of age, the villus height/crypt depth (V/C) ratio in the duodenum of Group was extremely significantly greater than that of Group (P<0.01) and significantly greater than that of Groups and (P<0.05), and the V/C ratio in the jejunum of Group at 42 days of age was extremely significantly greater than that of Groups , , and (P<0.01). 2) At 21 days of age, maltase activity in the duodenum and jejunum and sucrase activity in the jejunum were extremely significantly higher in Group than in Groups , , and (P<0.01); at 42 days of age, sucrase activity in the jejunum was extremely significantly higher in Group than in Groups and (P<0.01) and significantly higher than in Group (P<0.05). 3) At 42 days of age, the mRNA expression abundance of maltase-glucoamylase (MGA) in the jejunum of Group

was extremely significantly higher than that of Groups and (P<0.01). It was concluded that during the early (1-21 days of age) and middle (22-42 days of age) stages, xylanase supplementation could improve intestinal mucosal morphology, promote the secretion of disaccharidases and their gene expression in broilers, and the combined xylanase exhibited superior effects compared with single xylanases, demonstrating a synergistic effect of efficient catalysis.

Full Text

Effects of Different Sources of Xylanases and Their Combination on Intestinal Morphology, Disaccharidase Activities, and Gene Expression in Broilers

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Abstract

This experiment investigated the effects of different sources of xylanases and their combination on intestinal mucosal morphology, disaccharidase activities, and gene expression in yellow-feathered broilers. A total of 1,560 one-day-old healthy male yellow-feathered broilers were randomly allocated to 4 groups with 6 replicates per group and 65 broilers per replicate. Group I (control) received a low-energy, low-protein wheat-soybean basal diet, while groups II, III, and IV received the basal diet supplemented with 4,000 U/kg of xylanase 1# from *Aspergillus niger*, xylanase 2# from *Trichoderma koningii*, and a combined xylanase (xylanase 1#:xylanase 2# = 1:1), respectively. The 63-day trial period included tissue sampling at 21, 42, and 63 days of age, with two broilers selected from each replicate for analysis of intestinal mucosal morphology, disaccharidase activities, and disaccharidase gene mRNA expression abundances. The results demonstrated: (1) At 21 days, group IV showed significantly lower jejunal crypt depth compared to group I (P<0.05). At 42 days, group IV exhibited significantly greater villus height/crypt depth (V/C) values in the duodenum compared to group I (P<0.01) and groups II and III (P<0.05), with jejunal V/C values in group IV also significantly higher than all other groups (P<0.01). (2) At 21 days, maltase activities in duodenum and jejunum and jejunal sucrase activity were significantly higher in group IV than in groups I, II, and III (P<0.01). At 42 days, jejunal sucrase activity in group IV was significantly higher than in groups I and II (P<0.01) and group III (P<0.05). (3) At 42 days, jejunal maltase-glucoamylase (MGA) mRNA expression abundance in group IV was significantly higher than in groups I and II (P<0.01). These findings indicate

that xylanase supplementation improves intestinal mucosal morphology and promotes disaccharidase secretion and gene expression during early (1-21 days) and middle (22-42 days) growth stages, with the combined xylanase demonstrating superior efficacy compared to single enzymes, exhibiting an efficient catalytic synergistic effect.

Keywords: broilers; combined xylanase; mucosal morphology; disaccharidase; gene expression

Introduction

Cereal-based diets contain non-starch polysaccharides (NSP) such as xylan, whose antinutritional effects have long been recognized. Supplementing animal diets with NSP-degrading enzymes like xylanase can disrupt plant cell walls, enhance utilization of nutrients including starch and protein, reduce digesta viscosity to decrease disease incidence, and mitigate NSP-induced damage to intestinal mucosa while improving mucosal morphology. Cereal diets are also rich in starch, whose partial hydrolysis products—including maltose, maltotriose, and limit dextrins—cannot be directly absorbed by the intestine and must be hydrolyzed by mucosal disaccharidases into monosaccharides for absorption. Dietary carbohydrate content directly influences small intestinal disaccharidase hydrolysis, conferring strong adaptability to these enzymes.

Previous studies on single xylanase and xylanase-containing enzyme complexes have reported inconsistent effects on broiler intestinal morphology due to multiple factors influencing enzyme efficacy and resulting in varied production responses. To address this issue, Feng Dingyuan proposed the concept of combined enzymes, defined as enzyme preparations comprising two or more complementary enzymes selected from different sources and characteristics that catalyze hydrolysis of the same substrate through synergistic action. This study investigated the effects of different xylanase sources and their combination on broiler intestinal mucosal morphology, disaccharidase activities, and gene expression to explore the relationship between combined xylanase and intestinal mucosal morphology as well as carbohydrate digestion.

Materials and Methods

1.1 Experimental Animals and Design A total of 1,560 one-day-old healthy male Xinxing yellow-feathered broilers with similar initial body weight [(38±2) g] were randomly allocated to 4 dietary treatments with 6 replicates per treatment and 65 broilers per replicate. Group I (control) received a low-energy, low-protein wheat-soybean basal diet, while groups II, III, and IV received the basal diet supplemented with 4,000 U/kg of xylanase 1# from *Aspergillus niger*, xylanase 2# from *Trichoderma koningii*, and a combined xylanase (xylanase

1#:xylanase 2# = 1:1), respectively. The basal diet composition and nutrient levels are presented in . The 63-day experimental period allowed ad libitum access to feed and water.

1.2 Sample Collection At 21, 42, and 63 days of age, two broilers from each replicate were randomly selected, exsanguinated via carotid artery without feed withdrawal, and euthanized. Mid-segment samples of duodenum and jejunum were collected. Intestinal contents were gently rinsed with phosphate-buffered saline (PBS), cut into approximately 1 cm segments, and fixed. The intestines were then longitudinally opened, washed with ice-cold PBS, and blotted dry. Mucosal samples from mid-duodenum and mid-jejunum were scraped, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

1.3.1 Intestinal Mucosal Morphology Measurement Paraffin-embedded intestinal sections were stained with hematoxylin-eosin and examined using light microscopy with morphometric image analysis to measure villous height (VH), crypt depth (CD), and villous width (VW), and to calculate the villus height/crypt depth (V/C) ratio according to Frankel et al. Each parameter was measured three times and averaged. Villous height was defined as the vertical distance from the villus apex to the crypt opening; villous width as the maximum width of the villus; and crypt depth as the vertical distance from the crypt opening to the crypt base.

1.3.2 Intestinal Mucosal Biochemical Indicators Approximately 0.5 g of intestinal mucosa was homogenized in 4 mL of 0.1 mol/L maleic acid buffer (pH 6.8) on ice, incubated overnight at 4°C, and centrifuged at 3,000 rpm for 10 min at 4°C. The supernatant was used to determine mucosal glucose content and disaccharidase activities. Disaccharidase activity (U/g) was calculated as Xn/30a, where X represents glucose released (mol/L), a represents glucose release per disaccharide (2 for maltose, 1 for sucrose and lactose), n represents sample dilution factor, and 30 represents reaction time (min).

1.3.3 Intestinal Mucosal Disaccharidase mRNA Expression Abundance 1.3.3.1 Total RNA Extraction and Primer Design

Total RNA was extracted from intestinal mucosa using Trizol reagent (Tiangen Biotech, Beijing) according to manufacturer instructions. RNA concentration and purity were determined using a nucleic acid-protein analyzer (GERMAN BiopHotometer 6131, Eppendorf, Germany), and integrity was verified by agarose gel electrophoresis. After DNase I treatment, reverse transcription was performed using M-MLV reagent (Promega, USA). The resulting cDNA was stored at -30°C. Primers for chicken sucrase-isomaltase (SI), maltase-glucoamylase (MGA), and the reference gene -actin were designed using Primer 5.0 software and synthesized by Shanghai Sangon Biotech. Primer parameters are listed in .

1.3.3.2 Disaccharidase Gene mRNA Expression Abundance Measurement

Real-time quantitative PCR was used to determine disaccharidase gene mRNA expression abundance. Reaction conditions were: 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 58°C for 15 s, and 72°C for 15 s, with a final cycle of 95°C for 1 min, 58°C for 30 s, and 95°C for 30 s. The reaction system is shown in . Target gene mRNA expression abundance was calculated using the 2^-ΔCt method, where ΔCt = (target gene Ct - reference gene Ct).

1.4 Statistical Analysis Data were analyzed using SPSS 17.0 software. One-way ANOVA was performed on intestinal mucosal morphology indices, biochemical indicators, and disaccharidase gene mRNA expression abundances at different ages and intestinal segments, followed by LSD multiple comparisons. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effects of Different Xylanase Sources and Their Combination on Intestinal Mucosal Morphology At 21 days of age, duodenal villous width was significantly greater in groups I, III, and IV compared to group II ($P<0.01$), with group IV significantly greater than group I ($P<0.05$). Duodenal crypt depth was significantly lower in groups II, III, and IV compared to group I ($P<0.01$), while duodenal V/C values were significantly higher in group III compared to group I ($P<0.01$) and in groups II and IV compared to group I ($P<0.05$). Jejunal villous height was significantly higher in group IV compared to group I ($P<0.05$), with jejunal crypt depth significantly lower in group IV compared to group I ($P<0.05$). Jejunal V/C values were significantly higher in groups II and IV compared to group I ($P<0.01$) and in group IV compared to group III ($P<0.05$).

At 42 days of age, duodenal crypt depth was significantly lower in groups II and IV compared to group I ($P<0.01$) and group III ($P<0.05$), while duodenal V/C values were significantly higher in group IV compared to group I ($P<0.01$) and groups II and III ($P<0.05$). Jejunal villous height was significantly higher in group IV compared to group I ($P<0.01$) and group II ($P<0.05$), with group III also significantly higher than group I ($P<0.05$). Jejunal crypt depth was significantly lower in group IV compared to groups I and III ($P<0.05$), and jejunal V/C values were significantly higher in group IV compared to all other groups ($P<0.01$), with group III significantly higher than group I ($P<0.05$) and group II ($P<0.01$).

At 63 days of age, no significant differences were observed among groups for duodenal and jejunal villous height, villous width, or V/C values ($P>0.05$). Duodenal crypt depth also showed no significant differences among groups ($P>0.05$),

while jejunal crypt depth was significantly lower in groups II, III, and IV compared to group I ($P<0.01$). These results are summarized in .

2.2.1 Effects on Intestinal Mucosal Glucose Content At 21 days of age, duodenal mucosal glucose content showed no significant differences among groups ($P>0.05$). However, jejunal glucose content was significantly higher in groups III and IV compared to groups I and II ($P<0.01$), with group III showing the highest value and group I the lowest. At 42 days of age, duodenal glucose content remained similar across groups ($P>0.05$), while jejunal glucose content was significantly higher in group IV compared to groups I and II ($P<0.05$). At 63 days of age, duodenal glucose content again showed no significant differences ($P>0.05$), but jejunal glucose content was significantly higher in group II compared to group I ($P<0.01$) and in group III compared to group I ($P<0.05$). These findings are presented in .

2.2.2 Effects on Intestinal Mucosal Maltase Activity At 21 days of age, duodenal maltase activity was significantly higher in group IV compared to all other groups ($P<0.01$), with group III significantly higher than group I ($P<0.01$) and group II significantly higher than group I ($P<0.05$). Jejunal maltase activity at 21 days was also significantly higher in group IV compared to groups I, II, and III ($P<0.01$), with group II significantly higher than group I ($P<0.01$). At 42 days of age, duodenal maltase activity was significantly higher in groups II and IV compared to group I ($P<0.01$), with group IV significantly higher than group III ($P<0.05$), while jejunal maltase activity showed no significant differences among groups ($P>0.05$). At 63 days of age, duodenal maltase activity was significantly higher in groups III and IV compared to group II ($P<0.01$), with group IV significantly higher than group I ($P<0.05$), whereas jejunal maltase activity again showed no significant differences among groups ($P>0.05$). Detailed data are provided in .

2.2.3 Effects on Intestinal Mucosal Sucrase Activity At 21 days of age, duodenal sucrase activity was significantly higher in group IV compared to group I ($P<0.01$) and group III ($P<0.05$), while jejunal sucrase activity was significantly higher in group IV compared to groups I, II, and III ($P<0.01$), with group III significantly higher than group I ($P<0.01$) and group II significantly higher than group I ($P<0.05$). At 42 days of age, duodenal sucrase activity was significantly higher in group IV compared to group I ($P<0.05$), and jejunal sucrase activity was significantly higher in group IV compared to groups I and II ($P<0.01$) and group III ($P<0.05$), with group III also significantly higher than group I ($P<0.05$). At 63 days of age, no significant differences were observed among groups for either duodenal or jejunal sucrase activity ($P>0.05$). These results are detailed in .

2.4.1 Effects on Intestinal Mucosal MGA mRNA Expression Abundance At 21 days of age, duodenal MGA mRNA expression was significantly

higher in group IV compared to group I ($P<0.05$), while jejunal expression was significantly higher in group II compared to group I ($P<0.01$) and in groups III and IV compared to group I ($P<0.05$). At 42 days of age, duodenal MGA mRNA expression was significantly higher in groups III and IV compared to group I ($P<0.05$), and jejunal expression was significantly higher in group IV compared to groups I and II ($P<0.01$). At 63 days of age, no significant differences were observed among groups for either duodenal or jejunal MGA mRNA expression ($P>0.05$). These data are presented in .

2.4.2 Effects on Intestinal Mucosal SI mRNA Expression Abundance At 21 days of age, duodenal SI mRNA expression was significantly higher in group IV compared to group I ($P<0.01$) and in group II compared to group I ($P<0.05$), while jejunal expression showed no significant differences among groups ($P>0.05$). At 42 days of age, duodenal SI mRNA expression was significantly higher in groups II and IV compared to group I ($P<0.05$), and jejunal expression was significantly higher in groups III and IV compared to group I ($P<0.05$). At 63 days of age, duodenal SI mRNA expression was significantly higher in group IV compared to group II ($P<0.05$), while jejunal expression again showed no significant differences among groups ($P>0.05$). These findings are summarized in .

Discussion

3.1 Effects on Intestinal Mucosal Morphology Small intestinal villus morphology is critical for intestinal function and determines nutrient digestion and absorption capacity. Villus epithelial cells primarily perform absorptive functions, while crypt epithelial cells are mainly secretory. Net intestinal absorption depends on the V/C ratio, with increased V/C values indicating improved mucosal function, enhanced digestion and absorption, and accelerated growth and development. Non-starch polysaccharides in wheat-based diets can affect animal intestinal morphology, and xylanase supplementation has been shown to variably influence villous height, crypt depth, and V/C values. Yang et al. reported that xylanase supplementation in wheat-based diets did not significantly increase jejunal villous height in broilers but did reduce jejunal crypt depth. In the current study, xylanase supplementation in wheat-soybean diets increased duodenal and jejunal villous height and V/C values while reducing crypt depth in young (21 days) and growing (42 days) broilers, consistent with previous reports. The differential effects of xylanase on villous height, width, and crypt depth across different growth stages and intestinal segments may be attributed to variations in physiological stage and inherent enzyme characteristics.

3.2 Effects on Intestinal Mucosal Disaccharidase Activities The continuous process of epithelial cell senescence and shedding from villus tips 实质上 represents the secretion and release of disaccharidases and other oligosaccha-

ridases. After starch enters the small intestine, pancreatic amylase first produces -dextrins, maltotriose, and maltose, which are then hydrolyzed by brush border disaccharidases into monosaccharides for absorption by intestinal villi. Therefore, intestinal mucosal glucose content can reflect carbohydrate digestion capacity at the mucosal surface and indirectly indicate disaccharidase activity. Xylanase may influence disaccharidase activity and glucose content by reducing digesta viscosity and decreasing the thickness of the unstirred water layer. Soluble non-starch polysaccharides in cereal diets can affect intestinal disaccharidase activity, with maltase and sucrase being the predominant disaccharidases in non-mammalian intestinal mucosa. Xu Zirong et al. reported that piglets fed diets supplemented with NSP enzymes showed 38.46% and 40.00% higher jejunal maltase and sucrase activities, respectively, compared to unsupplemented controls. In the present study, xylanase substantially increased sucrase and maltase activities in young and growing broilers, aligning with previous findings. The mechanisms by which xylanase influences disaccharidase activity may include: (1) degrading arabinoxylan in wheat diets to provide more substrates for disaccharidases, and (2) reducing the viscosity of soluble NSP, thereby decreasing small intestinal digesta viscosity and facilitating access of disaccharides like maltose and sucrose to hydrolytic sites.

3.3 Effects on Intestinal Mucosal Disaccharidase Gene Expression
Disaccharidase gene mRNA expression abundance in animal intestinal mucosa changes during development. Weng Meiqian et al. reported that lactase-phlorizin hydrolase (LPH) and SI mRNA were expressed in all intestinal segments of newborn rabbits, with expression markedly enhanced 15 days after complete weaning. However, Yadgary et al. observed no significant age-related increase in SI mRNA expression abundance in chick embryos aged 11-21 days. In the current study, measurement of MGA and SI mRNA expression abundances at three growth stages revealed no consistent pattern. Diet composition and hormones can influence intestinal disaccharidase gene expression, with Mochizuki et al. reporting increased jejunal maltase and glucoamylase activities in mice fed high-starch, low-fat diets. In this experiment, xylanase supplementation in wheat-soybean diets enhanced MGA and SI mRNA expression abundances in intestinal mucosa during early and middle growth stages.

3.4 Theoretical Basis and Significance of Combined Enzyme Design
Combined enzymes utilize catalytic synergism by selecting two or more complementary enzymes from different sources and characteristics that hydrolyze the same substrate. In this study, seven single xylanases from different sources were evaluated for their enzymatic properties, from which complementary xylanases 1# and 2# were selected and combined at different ratios. The optimal complementary model was then identified under simulated broiler gastrointestinal conditions. With increasing use of unconventional feed ingredients, highly efficient combined enzymes will offer greater advantages. Therefore, future de-

velopment of feed enzyme preparations should focus on research and application of more combined or complex enzyme products.

Conclusion

During early (1-21 days) and middle (22-42 days) growth stages, xylanase supplementation improves intestinal mucosal morphology and promotes disaccharidase secretion and gene expression in broilers. At equivalent activities, the two single xylanases produce similar effects, while the combined enzyme demonstrates superior efficacy, exhibiting an efficient catalytic synergistic effect.

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