

Effects of Multi-Enzyme Complex and Probiotics Supplementation in Sorghum-Based Diets on Growth Performance, Serum Antioxidant Indices, and Intestinal Morphology in Liangfeng Broiler Chickens: Postprint

Authors: Zhao Jianfei, HU Guili, Tang Qianing, Song Zehe, Fan Zhiyong, Congratulations

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of adding enzyme complex and probiotics to sorghum-based diets on growth performance, serum antioxidant indices, and intestinal structure in Liangfenghua broiler chickens. A total of 900 healthy 1-day-old Liangfenghua broiler chicks with similar body weight were selected and randomly divided into 5 groups, with 6 replicates per group and 30 birds per replicate. The control group was fed a basal diet, while the experimental groups were fed sorghum-based diet (sorghum group), sorghum-based diet + enzyme complex (sorghum + enzyme complex group), sorghum-based diet + probiotics (sorghum + probiotics group), and sorghum-based diet + enzyme complex + probiotics (sorghum + enzyme complex + probiotics group). The experimental period lasted 56 days, divided into an early phase (1-28 days of age) and a late phase (29-56 days of age). At the end of the experiment, growth performance, serum antioxidant indices, and intestinal morphology of the broilers were measured. The results showed: 1) During the early phase, the average daily feed intake (ADFI) and feed-to-gain ratio (F/G) of broilers in all experimental groups were significantly lower than those in the control group ($P < 0.05$); during the late phase and the entire experimental period (1-56 days of age), except for the sorghum + enzyme complex group, the ADFI and average daily gain (ADG) of broilers in the other experimental groups were significantly lower than those in the control group ($P < 0.05$). 2) Compared with the control group, serum total superoxide dismutase (T-SOD) activity in the sorghum group was significantly decreased on day 28 ($P < 0.05$), with no significant differences among the other groups ($P > 0.05$); on day 56, serum antioxidant indices showed no significant differences among all groups ($P > 0.05$). 3) On day 28, except

for the sorghum group, duodenal villus height and villus height-to-crypt depth ratio (V/C) values in the other experimental groups were significantly higher than those in the control group ($P < 0.05$); on day 56, villus height, crypt depth, and V/C values in the duodenum, jejunum, and ileum showed no significant differences between the experimental groups and the control group ($P > 0.05$). In conclusion, replacing corn with sorghum in the diet significantly reduced the growth performance of Liangfenghua broiler chickens, whereas adding enzyme complex to sorghum-based diets achieved growth performance comparable to that of the control group; the addition of enzyme complex and probiotics to sorghum-based diets had no adverse effects on intestinal morphology or serum antioxidant indices in Liangfenghua broiler chickens.

Full Text

Effects of Compound Enzyme and Probiotic Supplementation in Sorghum-Based Diets on Growth Performance, Serum Antioxidant Indices, and Intestinal Morphology of Liangfenghua Broilers

ZHAO Jianfei, HU Guili, TANG Qianning, SONG Zehe, FAN Zhiyong, HE Xi*

College of Animal Science and Technology, Hunan Agricultural University; Engineering Research Center of Feed Safety and Efficient Utilization, Ministry of Education; Hunan Co-Innovation Center for Animal Production Safety, Changsha 410128, China

Abstract

This experiment was conducted to investigate the effects of compound enzyme and probiotic supplementation in sorghum-based diets on growth performance, serum antioxidant indices, and intestinal morphology of Liangfenghua broilers. A total of 900 healthy one-day-old Liangfenghua male broilers with similar body weight were randomly allocated to five groups with six replicates per group and 30 broilers per replicate. The control group was fed a basal diet, while the experimental groups were fed a sorghum-based diet (sorghum group), sorghum-based diet + compound enzyme (sorghum + enzyme group), sorghum-based diet + probiotics (sorghum + probiotics group), and sorghum-based diet + compound enzyme + probiotics (sorghum + enzyme + probiotics group), respectively. The 56-day experiment was divided into an early stage (1-28 days) and a later stage (29-56 days). At the end of the experiment, growth performance, serum antioxidant indices, and intestinal morphology were evaluated. The results showed: 1) During the early stage, the average daily feed intake (ADFI) and feed-to-gain ratio (F/G) of broilers in all experimental groups were significantly lower than those in the control group ($P < 0.05$). During the later stage and the entire experimental period (1-56 days), the ADFI and average daily gain (ADG) of

broilers in all experimental groups except the sorghum + enzyme group were significantly lower than those in the control group ($P < 0.05$). 2) Compared with the control group, serum total superoxide dismutase (T-SOD) activity in the sorghum group was significantly decreased at 28 days ($P < 0.05$), with no significant differences observed among the remaining groups ($P > 0.05$). At 56 days, no significant differences in serum antioxidant indices were detected among all groups ($P > 0.05$). 3) At 28 days, the duodenal villus height and villus height-to-crypt depth ratio (V/C) of broilers in all experimental groups except the sorghum group were significantly higher than those in the control group ($P < 0.05$). At 56 days, no significant differences in villus height, crypt depth, or V/C values in the duodenum, jejunum, or ileum were observed among any groups ($P > 0.05$). In conclusion, replacing corn with sorghum in diets significantly reduced the growth performance of Liangfenghua broilers, whereas supplementation with compound enzyme in sorghum-based diets achieved growth performance comparable to the control group. The combined supplementation of compound enzyme and probiotics in sorghum-based diets had no adverse effects on intestinal morphological structure or serum antioxidant indices.

Keywords: sorghum; compound enzyme; probiotics; growth performance; antioxidant indices; intestinal morphology

Introduction

The rapid development of animal husbandry in China has driven a sharp increase in feed demand, making feed resource shortage a critical bottleneck constraining the industry's growth. Corn serves as the primary feed ingredient in China, but various factors have led to increasing scarcity and price volatility of feed corn. To ensure sustainable, stable, and healthy development of the livestock and feed industries, the rational utilization of unconventional feed resources has become a growing focus. In some countries, sorghum has been widely applied as an energy feed ingredient in animal production, though its use remains relatively limited domestically.

The presence of anti-nutritional factors and protein encapsulation in sorghum, such as phytic acid, tannins, and prolamin, affects the digestion and utilization of nutrients. Cowieson et al. investigated the effects of phytase and a xylanase-amylase-protease complex alone and in combination in sorghum-based diets, demonstrating that the combination significantly improved broiler growth performance. Avila et al. reported that supplementation with a non-starch polysaccharide (NSP) enzyme complex during the later growth stage significantly improved broiler ADG, ADFI, and F/G. However, few studies have reported on the combined use of compound enzymes and probiotics in sorghum-based diets. Therefore, this experiment aimed to investigate the effects of compound enzyme and probiotic supplementation in sorghum-based diets on growth performance, serum antioxidant indices, and intestinal morphology of Liangfenghua broilers,

providing a scientific basis for improved sorghum application in animal production.

1.1 Experimental Materials

The sorghum used in this experiment was imported from the United States and purchased from Yueyang Port. The compound enzyme was a sorghum-specific preparation provided by a Beijing company, containing tannase (2,000 U/g), xylanase (20,000 U/g), -mannanase (1,500 U/g), protease (3,000 U/g), and amylase (500 U/g). The probiotic was a *Bacillus* preparation containing *Bacillus subtilis* provided by a Shandong company, with viable bacteria content 2.97×10^1 CFU/g.

1.2 Experimental Design and Diets

A total of 900 one-day-old Liangfenghua male broilers were obtained from Hunan Xiangjia Animal Husbandry Co., Ltd. and randomly allocated to five groups according to the principle of consistent average body weight, with six replicates per group and 30 broilers per replicate. The experiment used a corn-soybean meal basal diet formulated according to NRC (1994) and the Chinese Chicken Feeding Standard (NY/T 33-2004) to meet broiler nutritional requirements. The feeding program was divided into two phases: early stage (1-28 days) and later stage (29-56 days). Diet composition and nutrient levels are presented in Table 1.

The control group received the basal diet, while experimental groups received: (1) sorghum-based diet (sorghum group), (2) sorghum-based diet + compound enzyme (sorghum + enzyme group), (3) sorghum-based diet + probiotics (sorghum + probiotics group), and (4) sorghum-based diet + compound enzyme + probiotics (sorghum + enzyme + probiotics group). During the early stage, the sorghum-based diet replaced 30% of corn in the basal diet with sorghum and included 200 g/t compound enzyme; during the later stage, it replaced 50% of corn and included 300 g/t compound enzyme. Probiotics were added at 100 g/t throughout the entire 56-day period. Broilers were fed mash diets ad libitum.

1.3 Management

Broilers were raised in multi-tier cage systems with temperature controlled by boiler heating (33 °C on day 1, 30-32 °C during week 1, 27-29 °C during week 2, 24-26 °C during week 3, and maintained at 20-21 °C thereafter). Continuous artificial lighting was provided with natural ventilation, and nipple drinkers supplied water. Mash feed was provided ad libitum with free access to water. Conventional immunization was implemented, and feed intake and health status were monitored daily.

1.4 Measurements

1.4.1 Growth Performance Throughout the experimental period, growth and health status were observed. Feed consumption was recorded per replicate, and body weight was measured at 28 and 56 days after 8 hours of fasting to calculate ADG, ADFI, and F/G for each group.

1.4.2 Serum Antioxidant Indices At 28 and 56 days, one broiler per replicate with body weight close to the group average was selected for blood collection via cervical venipuncture. Six milliliters of blood were placed in 10 mL tubes, allowed to clot at 37 °C for 30 minutes, then centrifuged at 3,000 rpm for 15 minutes to obtain serum, which was stored at -20 °C for subsequent analysis. Serum catalase (CAT), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were measured using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.3 Intestinal Morphology After slaughter, 1 cm segments were collected from the duodenal U-shaped bend, mid-jejunum, and mid-ileum, and fixed in 10% formalin solution for histological sectioning. Fixed samples were processed through washing, dehydration, clearing, paraffin infiltration, embedding, and trimming before cutting into 6 μ m sections. Sections were stained with hematoxylin-eosin (HE) and sealed with neutral resin. Multiple non-consecutive fields were examined under microscopes at 100 \times , 250 \times , and 400 \times magnification, with representative fields photographed at 100 \times to compare intestinal villus morphology among groups. Image analysis software was used to measure villus height (VH) and crypt depth (CD), and to calculate the villus height-to-crypt depth (V/C) ratio.

1.5 Data Analysis

Data were analyzed using SPSS 19.0 statistical software. One-way ANOVA was performed followed by Duncan's multiple comparison test. Differences were considered significant at $P < 0.05$. Results are expressed as "mean \pm standard deviation."

Results

2.1 Effects on Growth Performance

As shown in Table 2, during the early stage, the sorghum group exhibited significantly lower ADFI and F/G compared with the control group ($P < 0.05$). Compared with the sorghum group, the sorghum + enzyme, sorghum + probiotics, and sorghum + enzyme + probiotics groups showed no significant differences in ADFI ($P > 0.05$) but had significantly lower F/G ($P < 0.05$), with both ADFI and F/G significantly lower than the control group ($P < 0.05$). No significant differences in ADG were observed among groups ($P > 0.05$).

During the later stage and entire experimental period, the sorghum group showed significantly lower ADFI and ADG compared with the control group ($P < 0.05$). Compared with the sorghum group, the sorghum + enzyme group exhibited significantly higher ADFI and ADG ($P < 0.05$), reaching levels comparable to the control group ($P > 0.05$). No significant differences in F/G were detected among groups during these periods ($P > 0.05$).

2.2 Effects on Serum Antioxidant Indices

Table 3 presents the serum antioxidant indices. At 28 days, serum T-SOD activity in the sorghum group was significantly lower than in the control group ($P < 0.05$). Compared with the sorghum group, the sorghum + enzyme, sorghum + probiotics, and sorghum + enzyme + probiotics groups showed significantly reduced T-SOD activity ($P < 0.05$), but these values did not differ significantly from the control group ($P > 0.05$). No significant differences in serum CAT, GSH-Px activities, MDA content, or T-AOC were observed among groups at either 28 or 56 days ($P > 0.05$).

2.3 Effects on Intestinal Morphology

Table 4 summarizes the intestinal morphological parameters. At 28 days, the sorghum group showed no significant differences in duodenal villus height or V/C ratio compared with the control group ($P > 0.05$). However, the sorghum + enzyme, sorghum + probiotics, and sorghum + enzyme + probiotics groups exhibited significantly greater duodenal villus height and V/C values compared with both the control and sorghum groups ($P < 0.05$). At 28 and 56 days, no significant differences were detected among groups in duodenal crypt depth or in any jejunal or ileal parameters including villus height, crypt depth, or V/C values ($P > 0.05$).

2.4 Effects on Economic Benefit

Table 5 shows the economic analysis. The sorghum + enzyme group demonstrated the best economic benefit compared with the control group, while all other experimental groups showed lower economic returns. These results indicate that when corn prices are high and sorghum prices are relatively low, supplementing sorghum-based diets with compound enzyme can effectively improve the economic efficiency of broiler production.

Discussion

3.1 Effects on Growth Performance

Feed intake serves as a fundamental measure of nutrient consumption in animals, as only nutrients exceeding maintenance requirements contribute to production. Therefore, feed intake represents a critical factor affecting animal productivity. The current results demonstrate that ADFI decreased in experimental groups

during the early stage, indicating that dietary sorghum inclusion reduced feed intake, likely due to the bitter taste of tannins impairing palatability. Although ADG did not differ significantly among groups, the sorghum + enzyme and sorghum + probiotics groups showed increasing trends. The significantly improved F/G in the sorghum + enzyme, sorghum + probiotics, and sorghum + enzyme + probiotics groups indicates that these supplements promoted early-stage growth. Wang et al. reported that *Bacillus* supplementation improved intestinal microflora and enhanced digestion, absorption, and live weight in chicks. In the early stage, replacing 30% of corn with sorghum significantly reduced ADFI and F/G without affecting ADG, suggesting that this level of substitution is feasible and may even promote early growth performance. However, the combination of compound enzyme and probiotics did not increase feed intake; in fact, the combined supplementation group showed the lowest intake. This may be attributed to improved energy utilization efficiency, indirectly reducing feed consumption. Alternatively, the rapid early growth rate of broilers, coupled with immature digestive systems and insufficient endogenous enzyme secretion, may limit nutrient digestion and absorption, constraining early growth. Dietary supplementation with enzymes and probiotics can produce nutrients, increase amylase and protease activities, provide enzymes not secreted endogenously, and enhance immune function, thereby improving digestive capacity and nutrient utilization. The current study found that combined supplementation significantly improved F/G. Collectively, early-stage data indicate that 30% corn replacement with sorghum promoted growth performance, with further enhancement from enzyme and probiotic supplementation, though combined addition may reduce palatability. The enzyme-only group performed best, possibly due to tannase activity mitigating the anti-nutritional effects of tannins.

Across the entire experimental period, the sorghum + enzyme group demonstrated optimal growth performance. Although F/G did not differ significantly among groups, all experimental groups showed numerically lower F/G than the control, indicating that corn replacement with sorghum improved feed efficiency. Jiraphocakul et al. found that *Bacillus subtilis* supplementation did not affect weight gain or F/G in turkeys. However, research on Wuding chicks fed different concentrations of compound microecological preparations showed varying degrees of growth performance improvement. The current results demonstrate that compound enzyme supplementation in sorghum-based diets enhanced broiler growth performance, suggesting that further optimization of enzyme and probiotic combinations could yield even better results.

3.2 Effects on Intestinal Morphology

Normal intestinal structure and function provide the biological foundation for growth and nutrient absorption. Villus height, crypt depth, and villus surface area serve as important indicators of intestinal digestive and absorptive capacity. Increased villus height enlarges the contact area with nutrients, enhancing

absorption and directly relating to growth performance. Crypt depth reflects cell generation rate, with continuous cell migration and differentiation from crypt bases to villus tips replacing normal epithelial shedding. Reduced crypt depth indicates decreased cell generation rate but increased maturation rate of enterocytes, enhancing nutrient absorption function. The V/C ratio comprehensively reflects intestinal functional status, with higher values indicating improved digestive and absorptive capacity.

Shi reported that dietary supplementation with *Bacillus licheniformis* and xylo-oligosaccharides increased jejunal villus height and reduced crypt depth, though differences were not significant compared with the control. Wu found that fructooligosaccharides, NSP enzymes (xylanase + -mannanase), and *Bacillus subtilis* all improved intestinal morphology in Guangxi Yellow-feathered chickens by increasing villus height and V/C values while reducing intestinal wall thickness. Lei demonstrated that xylanase supplementation in wheat-based diets reduced duodenal wall thickness and increased ileal villus height without affecting jejunal morphology. The current findings indicate that compound enzyme and probiotic supplementation in sorghum-based diets improved small intestinal morphology and promoted nutrient absorption, thereby enhancing growth performance.

3.3 Effects on Serum Antioxidant Indices

Animal antioxidant systems comprise non-enzymatic components (selenium, glutathione, vitamin E, vitamin C) and enzymatic components (GSH-Px, SOD, CAT). Antioxidant capacity can be evaluated by measuring enzyme activities in serum and liver, as well as peroxidation products such as MDA, hydroxyl, and carbonyl groups. Increased free radicals trigger oxidative reactions, stimulating antioxidant enzyme production to eliminate excess radicals. Changes in antioxidant enzyme levels indirectly reflect oxidative stress processes.

MDA, the end product of lipid peroxidation, indicates the degree of lipid peroxidation and cellular damage. SOD prevents free radical damage, and its activity reflects the capacity to scavenge free radicals. GSH-Px eliminates hydrogen peroxide and lipid radicals, while CAT catalyzes hydrogen peroxide decomposition to prevent meat oxidation. The current results demonstrate that sorghum-based diets reduced antioxidant capacity during the early stage but had minimal impact during the later stage.

Conclusion

Replacing corn with sorghum in diets reduced the growth performance of Liangfenghua broilers and affected early-stage intestinal morphology. Supplementation with compound enzyme and probiotics in sorghum-based diets had no adverse effects on intestinal morphological structure or serum antioxidant indices. Notably, compound enzyme supplementation in sorghum-based diets achieved growth performance comparable to the control group while providing

the best economic benefit.

References

- [1] Zhang Zhenlei, Lu Yu. Development and utilization of unconventional feed resources[J]. *Feed China*, 2017(01): 50-51.
- [2] Mi Yan, Zhu Linna, Chen Guoying, et al. Research and application of enzyme preparations in sorghum-based diets[J]. *Guangdong Feed*, 2015, 24(8): 31-33.
- [3] Cowieson A J, Adeola O. Carbohydrases, protease, and phytase have an additive beneficial effect in nutritionally marginal diets for broiler chicks[J]. *Poultry Science*, 2005, 84(12): 1860.
- [4] Avila E, Arce J, Soto C, et al. Evaluation of an enzyme complex containing nonstarch polysaccharide enzymes and phytase on the performance of broilers fed a sorghum and soybean meal diet[J]. *Journal of Applied Poultry Research*, 2012, 21(2): 279-286.
- [5] Yang Feng. *Animal Nutrition*[M]. Beijing: China Agriculture Press, 2004.
- [6] Guo Aiwei, Zhou Jielong, Zhu Jing, et al. Effects of compound Chinese herbal medicine replacing antibiotics on production performance and substance metabolism in broilers[J]. *Acta Agriculturae Universitatis Jiangxiensis*, 2008, 30(6): 977-980.
- [7] Wang Zheng, Zhang Dawei, Qi Changhai, et al. Effects of different supplementation levels of *Bacillus* preparation on intestinal microflora and some physiological indices of laying hens during the brooding period[J]. *Chinese Journal of Animal Science*, 2017, 53(05): 156-159.
- [8] Xi Haibo, Yao Junhu, Chen Xinke. Effects of compound enzyme preparation on broiler production performance[J]. *Heilongjiang Animal Science and Veterinary Medicine*, 2006(1): 46-47.
- [9] Jiraphocakul S, Sullivan T W, Shahani K M. Influence of a dried *Bacillus subtilis* culture and antibiotics on performance and intestinal microflora in turkeys[J]. *Poultry Science*, 1990, 69(11): 1966-1973.
- [10] Xu Jiafu. Effects of compound microecological preparation on growth performance and some immune indices of Wuding chicks[D]. Master's thesis. Kunming: Yunnan Agricultural University, 2017.
- [11] Varel V H, Robinson I M, Pond W G. Effect of dietary copper sulfate, Aureo SP250, or clinoptilolite on ureolytic bacteria found in the pig large intestine[J]. *Applied & Environmental Microbiology*, 1987, 53(9): 2009.
- [12] Caspary W F. Physiology and pathophysiology of intestinal absorption[J]. *American Journal of Clinical Nutrition*, 1992, 55(1): 299S-308S.
- [13] Wang Zixu. Study on the effects of zinc-selenium interaction on intestinal mucosal structure and mucosal immune-related cells in broilers[D]. Master's

thesis. Beijing: China Agricultural University, 2003.

[14] Liu Yanqiang, Han Zhengkang. Effects of crude enzyme supplementation in barley-based diets on metabolic hormones in chicken peripheral blood[J]. *Chinese Journal of Veterinary Science*, 1998, 18(6): 577-580.

[15] Shi Ning. Effects of *Bacillus licheniformis*, xylo-oligosaccharides, and lactic acid on intestinal villus tissue and microflora in broilers[D]. Master' s thesis. Zhengzhou: Henan University of Technology, 2010.

[16] Wu Tianpei. Effects of fructooligosaccharides, NSP enzymes, and probiotics on production performance, serum indices, intestinal morphology, and microflora in Yellow-feathered chickens[D]. Master' s thesis. Nanning: Guangxi University, 2012.

[17] Lei Li. Study on degradation patterns of non-starch polysaccharides by xylanase and its effects on intestinal morphology in broilers fed wheat-based diets[D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2007.

[18] Temple N J. Antioxidants and disease: more questions than answers[J]. *Nutrition Research*, 2000, 20(3): 449-459.

[19] Jiang Buyun. Study on the effects of plant polyphenols on antioxidant capacity and meat quality in Yellow-feathered broilers[D]. Master' s thesis. Changsha: Hunan Agricultural University, 2014.

[20] Gao Tianshuang. Effects of different proportions of sorghum diets on production performance and digestion-metabolism in lambs[D]. Master' s thesis. Harbin: Northeast Agricultural University, 2014.

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

Source: ChinaXiv –Machine translation. Verify with original.