

Effects of Different Diets and Additives on Growth Performance, Intestinal Structure and Function in Broiler Chickens: Postprint

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Abstract

This study aimed to investigate the effects of different diet types and additives on growth performance, intestinal structure, and function in broiler chickens.

The experiment employed a 4×3 two-factor completely randomized design. The four diets were: antibiotic diet, antibiotic-free diet, low-protein diet, and barley diet. The three additives were: yeast culture (XPC), glutamine (Gln), and soy iso flavone (ISF). A total of 7921 19-day-old Arbor Acres broiler chickens were randomly assigned to 12 groups, with 6 replicates per group and 11 chickens per replicate. 1) The addition of XPC, Gln, and ISF to different diet types had no significant effects ($P > 0.05$) on average daily gain (ADG), average daily feed intake (ADFI), feed-to-gain ratio (F/G), or body weight (BW) of 42-day-old broiler chickens. However, regarding diet type, compared with the protein diet, significantly decreased BW, ADG, and ADFI ($P < 0.05$) and significantly increased F/G ($P < 0.05$). The antibiotic diet significantly decreased F/G ($P < 0.05$). There was no significant interaction effect ($P > 0.05$) between diet type and additives on growth performance. 2) Compared with Gln supplementation, ISF supplementation in 19-day-old broiler chickens ($P < 0.05$). XPC supplementation in the low-protein diet had a significant improving effect on ileal morphology compared with the other two additives ($P < 0.05$). Compared with XPC, ISF supplementation in the barley diet significantly improved ileal morphology in 19-day-old broiler chickens ($P < 0.05$). Except for ileal crypt depth in 35-day-old broiler chickens, there was a significant interaction effect ($P < 0.05$) between diet type and additives on ileal morphology. 3) XPC supplementation in the barley diet significantly increased ileal IgA levels in 35-day-old broiler chickens compared with Gln and ISF ($P < 0.05$). Compared with ISF, XPC supplementation in the antibiotic diet significantly increased ileal interleukin-10 (IL-10) and immunoglobulin A (IgA) levels in 19-day-old broiler chickens ($P < 0.05$), while Gln supplementation significantly increased ileal IgA levels in 35-day-old broiler chickens ($P < 0.05$). XPC supplementation in the low-protein diet significantly increased ileal IgA levels in 19-day-old broiler chickens compared with the other two additives ($P < 0.05$). ISF supplementation in the antibiotic-free diet significantly increased ileal IL-10 and IgA levels in 35-day-old broiler chickens compared with XPC ($P < 0.05$),

whereas ISF supplementation in the barley diet significantly decreased ileal IL-10 levels in 35-day-old broiler chickens compared with XPC ($P < 0.05$). There was a significant interaction effect ($P < 0.05$) between diet type and additives on ileal IL-10 and IgA levels. 4) XPC supplementation in the antibiotic diet significantly reduced nitrogen and phosphorus residual rates in feces of 19-day-old broiler chickens compared with the other two additives ($P < 0.05$). XPC supplementation in the antibiotic-free diet significantly increased nitrogen and phosphorus residual rates in feces of 19-day-old broiler chickens and phosphorus residual rate in feces of 35-day-old broiler chickens compared with the other two additives ($P < 0.05$). XPC supplementation in the low-protein diet significantly reduced nitrogen residual rate in feces of 19-day-old broiler chickens compared with Gln ($P < 0.05$). Gln supplementation in the barley diet significantly reduced nitrogen and phosphorus residual rates in feces of 19-day-old broiler chickens compared with the other two additives ($P < 0.05$). There was a significant interaction effect ($P < 0.05$) between diet type and additives on nitrogen and phosphorus residual rates. In conclusion, the addition of three additives to four diet types had no significant effect on growth performance of 1- to 42-day-old broiler chickens. The low-protein diet with fixed energy-to-protein ratio reduced growth performance in broiler chickens. XPC supplementation in the low-protein diet improved ileal structural morphology. The addition of different additives to different diet types showed trends toward improving ileal immune status and reducing nitrogen and phosphorus residual rates in feces.

Full Text

Effects of Different Types of Diets and Additives on Growth Performance, Gut Structure and Functions of Broilers

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Abstract: This experiment was conducted to investigate the effects of different diet types and additives on growth performance, gut structure, and function in broiler chickens. A 4×3 factorial arrangement was employed in a completely randomized design, with four diet types (freediet, low-protein diet, and barley diet) and three additive treatments (yeast culture [XPC], glutamine [Gln], and ISF) in a 42-day-old Arbor Acres broilers were randomly allocated to 12 groups with 6 replicates of 11 birds each for a 42-day feeding trial. The results showed that: (1) Supplementation of XPC, Gln, and ISF to the four different diet types significantly increased the feed-to-gain ratio (F/G), or body weight (BW) at 42 days of age ($P > 0.05$). However, among diet types, the low-protein diet significantly reduced BW, ADG, and ADFI ($P < 0.05$) and increased F/G ($P < 0.05$) compared with the other three diets, while the antibiotic diet significantly decreased F/G ($P < 0.05$). No significant interaction between diet type and additive was observed for growth performance ($P > 0.05$). (2) In the antibiotic diet, ISF supplementation significantly improved ileal morphology at 19 days compared with

0.05). In the low-protein diet, XPC supplementation significantly improved ileal morphology compared with the other additives ($P < 0.05$). In the barley diet, ISF supplementation significantly improved ileal morphology at 19 days compared with XPC ($P < 0.05$). A significant interaction between diet type and additive was observed for ileal morphology ($P < 0.05$), except for crypt depth at 35 days. (3) In the barley diet, XPC supplementation significantly increased ileal IFN- γ level at 35 days compared with Gln and ISF ($P < 0.05$). In the antibiotic diet, XPC supplementation significantly increased interleukin-10 (IL-10) and immunoglobulin A (IgA) levels at 19 days compared with ISF ($P < 0.05$), while Gln supplementation significantly increased IgA level at 35 days ($P < 0.05$). In the low-protein diet, XPC supplementation significantly increased ileal IgA level at 19 days compared with the other additives ($P < 0.05$). In the antibiotic-free diet, ISF supplementation significantly increased IL-10 and IgA levels at 35 days compared with XPC ($P < 0.05$), while ISF supplementation in the barley diet significantly decreased IL-10 level at 35 days compared with XPC ($P < 0.05$). A significant interaction between diet type and additive was observed for ileal IL-10 and IgA levels ($P < 0.05$). (4) In the antibiotic diet, XPC supplementation significantly reduced fecal nitrogen and phosphorus retention at 19 days compared with Gln and ISF ($P < 0.05$). In the antibiotic-free diet, XPC supplementation significantly increased fecal nitrogen and phosphorus retention at 19 days and phosphorus retention at 35 days compared with the other additives ($P < 0.05$). In the low-protein diet, XPC supplementation significantly reduced nitrogen retention at 19 days compared with Gln ($P < 0.05$). In the barley diet, Gln supplementation significantly reduced nitrogen and phosphorus retention at 19 days compared with XPC and ISF ($P < 0.05$). A significant interaction between diet type and additive was observed for nitrogen and phosphorus retention ($P < 0.05$). In conclusion, supplementation of the three additives to the four diet types did not significantly affect growth performance from 1 to 42 days of age. The low-protein diet with fixed energy-to-protein ratio reduced broiler growth performance, while XPC supplementation improved ileal morphology in the low-protein diet. Different additives in different diet types showed tendencies to improve ileal immune status and reduce fecal nitrogen and phosphorus excretion.

Keywords: broilers; growth performance; intestine; diet types; additives

Introduction

Diet type has become a significant factor influencing digestive tract structure and function in broiler production. Previous research demonstrated that antibiotic diets reduced muscularis mucosa thickness and villus surface area while decreasing villus height in the ileum compared with control diets. Studies have also shown that low-protein diets with constant energy-to-protein ratios impair growth performance, while dietary protein levels affect microbial growth in the lower digestive tract, potentially altering gut health. Barley, as a lower-quality feed ingredient compared with corn, has been found to significantly reduce body weight and increase feed-to-gain ratio; additionally, high levels of non-starch

polysaccharides in barley increase intestinal viscosity and impede nutrient absorption, ultimately compromising gut health.

Beyond diet type, dietary additives such as yeast products, glutamine, and soybean isoflavones positively influence intestinal health. These three additives have been reported to protect intestinal structure, promote repair after injury, and improve gut immune status. Yeast products, including live yeast, yeast extract, and yeast cell wall, have over a century of application in animal nutrition, with live yeast protecting intestinal mucosa and preventing pathogen invasion. Although glutamine is a non-essential amino acid, research indicates that its requirements cannot be met during stress or infection, and it promotes better restoration of damaged intestinal mucosa. Soybean isoflavones exhibit potential estrogenic activity, with genistein, daidzein, and glycitein being the most important bioactive forms. Studies have reported that isoflavones improve growth performance and antioxidant capacity while enhancing intestinal immune status under lipopolysaccharide stress. Therefore, this study selected XPC, Gln, and ISF as additives known to improve the digestive tract in broilers and compared their effects on growth performance, gut structure, and function across different diet types.

Materials and Methods

1.1 Experimental Materials

XPC was sourced from Diamond V (USA), L-Gln from Beijing Jiakangyuan Technology Development Co., Ltd. (98% purity), and ISF from Beijing Jiakangyuan Technology Development Co., Ltd. (40% total flavonoids content).

1.2 Experimental Animals and Diets

A 4×3 factorial completely randomized design was employed using 792 healthy one-day-old Arbor Acres male broilers with similar initial body weight [(37.58±0.55) g]. Birds were divided into 12 groups with 6 replicates of 11 chickens each. The four dietary treatments consisted of an antibiotic diet (A), an antibiotic-free diet (NA), a low-protein diet with fixed energy-to-protein ratio (LP), and a barley diet (B). Three additives were supplemented at 0.2% XPC, 0.6% Gln, and 10 mg/kg ISF across the four diets. Diets were formulated according to the Chinese Ministry of Agriculture industry standard (NY/T 33–2004) “Feeding Standard of Chickens.” Diet composition and nutrient levels are presented in .

1.3 Management

The 42-day trial employed cage rearing with temperature controlled according to the Arbor Acres Broiler Management Guide. Birds had ad libitum access to feed and water via nipple drinkers under 24-hour lighting. Vaccination occurred at 7 days (Newcastle disease and infectious bronchitis vaccine, eye and nasal

drop) and 14 days (infectious bursal disease vaccine, via drinking water). Bird health and feed consumption were monitored and recorded daily.

1.4 Measurements

1.4.1 Growth Performance Bird health was recorded daily throughout the trial. At 42 days, birds were weighed by replicate after feed withdrawal to determine body weight (BW), from which average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated.

1.4.2 Ileal Morphology At 19 and 35 days, one bird per replicate was randomly selected, fasted, weighed, and euthanized by electrical stunning. A 2-cm ileal segment was excised, rinsed with saline, and fixed in 4% paraformaldehyde for histological sectioning. Villus height and crypt depth were measured to calculate villus height-to-crypt depth ratio (V/C).

1.4.3 Immune Parameters At 19 and 35 days, one bird per replicate was euthanized, and a 4-cm ileal segment was collected, blotted dry, and snap-frozen in liquid nitrogen for analysis of interferon- γ (IFN- γ), interleukin-10 (IL-10), and immunoglobulin A (IgA) levels. IFN- γ was measured using a radioimmunoassay kit (HY-156, RIA KIT), IL-10 using an iodine [125 I] IL-10 radioimmunoassay kit (HY-10107, RIA KIT), and IgA using a chicken IgA assay kit (HY-754, KIT) with immunoturbidimetry.

1.4.4 Fecal Nitrogen and Phosphorus Retention Fresh feces were collected from days 17-19 and 33-35, pooled by replicate, mixed thoroughly, and sprayed with 10% hydrochloric acid for nitrogen fixation before storage at -20°C . Samples were heated at 105°C for 15 minutes, dried at 65°C for 72 hours, equilibrated for 24 hours, ground through a 40-mesh sieve, and stored sealed. Titanium dioxide content in diets and feces was determined by spectrophotometry. Crude protein (nitrogen) content was measured by combustion (Dumatherm, Gerhardt, Germany), and phosphorus content by molybdenum yellow colorimetry.

1.5 Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure in SPSS 19.0 (2007, SPSS Inc., Chicago, IL 60606-6307) for two-way ANOVA. Factor significance was tested using F-tests, and significant main effects were subjected to LSD multiple comparison tests. Significance was set at $P < 0.05$, and data are presented as mean \pm SD.

Results

2.1 Effects of Diet Types and Additives on Growth Performance

The effects of different diet types and additives on growth performance from 1 to 42 days are shown in . Supplementation of XPC, Gln, and ISF to the four different diets did not significantly affect ADG, ADFI, F/G, or BW at 42 days ($P > 0.05$). However, among diet types, the low-protein diet significantly reduced BW, ADG, and ADFI ($P < 0.05$) and increased F/G ($P < 0.05$) compared with the other three diets. The antibiotic diet significantly decreased F/G compared with other groups ($P < 0.05$). No significant differences were observed among the three additives for ADG, ADFI, F/G, or BW ($P > 0.05$), and no significant interaction existed between diet type and additive for growth performance ($P > 0.05$). All birds remained healthy throughout the trial with minimal mortality.

2.2 Effects of Diet Types and Additives on Ileal Morphology

The effects of different diet types and additives on ileal morphology at 19 and 35 days are presented in . In the antibiotic diet, XPC supplementation significantly increased ileal villus height at 19 days compared with Gln and ISF ($P < 0.05$), while ISF supplementation significantly reduced crypt depth and increased V/C ratio compared with Gln ($P < 0.05$). Gln supplementation significantly increased villus height at 35 days compared with ISF ($P < 0.05$). In the antibiotic-free diet, Gln supplementation significantly increased villus height at 19 days compared with the other additives ($P < 0.05$). In the low-protein diet, XPC supplementation significantly reduced crypt depth and increased villus height and V/C ratio at both 19 and 35 days compared with ISF and Gln ($P < 0.05$). In the barley diet, ISF supplementation significantly increased V/C ratio at 19 days compared with XPC ($P < 0.05$), with no other significant differences observed ($P > 0.05$). Among diet types, antibiotic and antibiotic-free diets significantly increased villus height at 19 days compared with the barley diet ($P < 0.05$), and the antibiotic-free diet showed higher V/C ratio at 19 days ($P < 0.05$). The low-protein diet significantly increased villus height and V/C ratio at 35 days compared with the other diets ($P < 0.05$). Among additives, ISF significantly reduced crypt depth at 19 days compared with XPC ($P < 0.05$), while XPC significantly increased villus height at 35 days compared with Gln and ISF ($P < 0.05$). A significant interaction between diet type and additive was observed for ileal morphology ($P < 0.05$), except for crypt depth at 35 days.

2.3 Effects of Diet Types and Additives on Ileal Immune Parameters

The effects of different diet types and additives on ileal immune parameters at 19 and 35 days are shown in . Barley diet supplemented with XPC significantly increased ileal IFN- γ level at 35 days compared with Gln and ISF ($P < 0.05$), while other treatments did not significantly affect IFN- γ levels at either age ($P > 0.05$). In the antibiotic diet, XPC supplementation significantly increased IL-10 and IgA levels at 19 days compared with ISF ($P < 0.05$), with IgA levels

higher than all other groups ($P < 0.05$). In the antibiotic-free diet, XPC and Gln supplementation significantly increased IL-10 level at 19 days compared with ISF ($P < 0.05$), while Gln and ISF supplementation significantly increased IgA level at 19 days and IL-10 level at 35 days compared with XPC ($P < 0.05$). In the low-protein diet, XPC supplementation significantly increased IgA level at 19 days compared with Gln and ISF ($P < 0.05$). In the barley diet, ISF supplementation significantly increased IL-10 level at 19 days compared with the other additives ($P < 0.05$), while XPC supplementation significantly increased IgA level at 19 days compared with ISF ($P < 0.05$). XPC and Gln supplementation significantly increased IL-10 level at 35 days compared with ISF ($P < 0.05$). Among diet types, antibiotic and antibiotic-free diets significantly increased IL-10 level at 19 days compared with low-protein and barley diets ($P < 0.05$). At 35 days, the antibiotic diet significantly reduced IL-10 level ($P < 0.05$) while increasing IgA level ($P < 0.05$). The barley diet significantly increased IgA levels at both ages compared with the antibiotic-free and low-protein diets ($P < 0.05$). Among additives, XPC significantly increased IL-10 and IgA levels at 19 days compared with ISF ($P < 0.05$), while Gln significantly increased IgA level at 35 days compared with the other additives ($P < 0.05$). No significant interaction was observed for IFN- γ level ($P > 0.05$), but significant interactions existed for IL-10 and IgA levels ($P < 0.05$).

2.4 Effects of Diet Types and Additives on Fecal Nitrogen and Phosphorus Retention

The effects of different diet types and additives on fecal nitrogen and phosphorus retention are presented in . In the antibiotic diet, XPC supplementation significantly reduced nitrogen and phosphorus retention at 19 days compared with Gln and ISF ($P < 0.05$), while ISF supplementation significantly reduced nitrogen and phosphorus retention at 35 days compared with XPC and Gln ($P < 0.05$). In the antibiotic-free diet, Gln and ISF supplementation significantly reduced nitrogen and phosphorus retention at 19 days and phosphorus retention at 35 days compared with XPC ($P < 0.05$). In the low-protein diet, XPC supplementation significantly reduced nitrogen retention at 19 days compared with Gln ($P < 0.05$). In the barley diet, Gln supplementation significantly reduced nitrogen and phosphorus retention at 19 days compared with XPC and ISF ($P < 0.05$). Among diet types, the antibiotic diet significantly reduced nitrogen and phosphorus retention at 19 days compared with the other diets ($P < 0.05$). The low-protein diet showed lower nitrogen retention at 35 days compared with the antibiotic-free and barley diets ($P < 0.05$), while the barley diet exhibited higher phosphorus retention than the antibiotic and antibiotic-free diets ($P < 0.05$). Among additives, Gln and ISF significantly reduced phosphorus retention at 19 days compared with XPC ($P < 0.05$), and ISF significantly reduced nitrogen and phosphorus retention at 35 days compared with XPC ($P < 0.05$). A significant interaction between diet type and additive was observed for nitrogen and phosphorus retention ($P < 0.05$).

Discussion

3.1 Effects of Diet Types and Additives on Growth Performance

Diet type and energy-to-protein ratio are primary determinants of broiler growth performance. This study confirmed that feeding broilers a low-protein diet with fixed energy-to-protein ratio reduced growth performance, consistent with previous reports. Researchers have demonstrated that suboptimal dietary energy and protein levels impair broiler performance, and low-protein diets with constant energy-to-protein ratios reduce growth performance. Among the four diet types, the antibiotic diet produced the lowest feed-to-gain ratio. Previous studies reported that chlortetracycline at 100 mg/kg did not significantly improve daily gain but enhanced feed conversion efficiency by 2.49% at 50 mg/kg. Under our experimental conditions, no significant differences were observed among the three additives for ADG, ADFI, F/G, or BW, and no significant interaction existed between diet type and additive for growth performance. Studies in turkeys showed that XPC did not affect weight gain or feed conversion from 3 to 15 weeks. Dietary Gln supplementation at 0-1.5% did not significantly affect ADFI or F/G in broilers, and researchers have speculated that endogenous Gln synthesis may be sufficient to maintain intestinal epithelial function in low-protein diets, thus supplemental Gln does not improve growth retardation or feed efficiency. Similarly, ISF supplementation in low-protein diets did not improve ADFI, ADFI, or feed conversion.

3.2 Effects of Diet Types and Additives on Ileal Morphology

Villus height, crypt depth, and V/C ratio are commonly used indicators of intestinal morphology and are associated with local or systemic inflammation. In this study, low-protein diet supplemented with XPC significantly increased ileal villus height and V/C ratio while reducing crypt depth at 19 days compared with ISF, and significantly increased villus height and V/C ratio at 35 days compared with both ISF and Gln. Previous research demonstrated that 0.25% XPC significantly increased ileal villus height and V/C ratio at 21 days. However, under heat stress conditions, dietary Gln supplementation at 0.25-1.00% did not significantly affect ileal structure. At 19 days, ISF supplementation in antibiotic and barley diets significantly increased V/C ratio compared with Gln or XPC. The gastrointestinal mucosa is continuously exposed to oxidants, mutagens, and reactive oxygen species from the intestinal lumen, making it highly sensitive to oxidative stress. Isoflavones have demonstrated potent antioxidant potential and may protect and maintain ileal morphology through antioxidant mechanisms.

3.3 Effects of Diet Types and Additives on Ileal Immune Parameters

IFN- γ , secreted by antigen-presenting cells and Th1 cells, increases tight junction permeability. IL-10 prevents intestinal disease by reducing cytokine production, preventing inflammatory responses, and inhibiting IFN- γ production. IgA

is secreted by B lymphocytes in the lamina propria and continuously released into the intestinal lumen. Previous studies reported that 0.25% XPC did not affect duodenal IgA levels at 21 days but increased IgA at 42 days compared with higher XPC levels. In vitro studies showed that XPC downregulated IFN- γ induced by IL-2 or phytohemagglutinin, activated B lymphocytes, and promoted Th2 pathways. Our study found that XPC supplementation in antibiotic diets significantly increased IL-10 and IgA levels at 19 days compared with ISF, and XPC in low-protein and barley diets significantly increased IgA levels compared with ISF. However, XPC in barley diet did not reduce IFN- γ level at 35 days. Glutamine has been shown to increase IgA levels in the esophagus after stress and promote IgA secretion in the intestine. In mice, glutamine enhanced IgA secretion, though 0.5% Gln did not affect blood IFN- γ levels. In our study, Gln supplementation in antibiotic-free diets significantly increased IL-10 level at 19 days but reduced IgA level at 35 days. In barley diets, Gln significantly reduced IFN- γ level at 35 days while increasing IL-10 level compared with ISF. Intestinal microorganisms play important roles in ISF metabolism, and ISF suppresses inflammatory responses by regulating pro-inflammatory cytokines such as IL-6. Studies have shown that ISF inhibits IFN- γ secretion from CD4⁺ T lymphocytes induced by lipopolysaccharide and suppresses mucosal immune responses. ISF may shift Th1/Th2 balance toward Th2 by reducing the IFN- γ /IL-10 ratio. In this study, ISF in antibiotic diets reduced IL-10 and IgA levels at 19 days and IgA at 35 days compared with XPC or Gln. However, ISF in antibiotic-free diets significantly increased IL-10 and IgA levels at 35 days, demonstrating significant immunomodulatory effects. In barley diets, ISF significantly reduced both IFN- γ and IL-10 levels at 35 days. These findings suggest that the immunomodulatory effects of different additives vary depending on diet type.

3.4 Effects of Diet Types and Additives on Fecal Nitrogen and Phosphorus Retention

The results showed that XPC supplementation in antibiotic diets significantly reduced nitrogen and phosphorus retention at 19 days compared with Gln and ISF, while Gln supplementation in barley diets significantly reduced nitrogen and phosphorus retention at 19 days compared with XPC and ISF. Previous studies reported that XPC did not improve phosphorus digestibility at 15 days but significantly increased it at 35 days at 0.50% and 0.75% supplementation levels. Other research demonstrated that 0.10-0.20% XPC significantly improved phosphorus digestibility. In this study, ISF supplementation in barley diets increased nitrogen and phosphorus retention at 19 days compared with Gln, which contradicts previous reports that genistein improved crude protein digestibility and reduced phosphorus retention under heat stress. In antibiotic-free diets, Gln and ISF significantly reduced nitrogen and phosphorus retention at 19 days and phosphorus retention at 35 days compared with XPC. Glutamine has been shown to improve barrier function in mice with colitis. In piglets, dietary Gln increased apparent digestibility of dry matter and crude protein by 9.06% and 4.77% at 10 days post-weaning and by 5.90% and 2.80% at 30 days. However, in

broilers, 1-3% Gln supplementation only numerically reduced nitrogen retention at 21 days without statistical significance.

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