Effects of Oxidized Fish Meal on Growth Performance, Digestive Tract Structure and Function in Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of oxidized fish meal protein on growth performance, gastrointestinal tract structure and function in broiler chickens. A total of 180 healthy 1-day-old male broiler chicks with similar body weight were randomly allocated into 3 groups, with 6 replicates per group and 10 chicks per replicate. The three groups were fed a control diet (CON group), a diet containing 2% normal fish meal (FM group), and a diet containing 2% oxidized fish meal (OFM group), respectively, for a 21-day experimental period. The results showed: 1) Compared with the CON and FM groups, the OFM group significantly decreased body weight and average daily gain ($P<0.05$), and significantly increased feed-to-gain ratio ($P<0.05$) in broiler chickens; 2) Compared with the CON and FM groups, broiler chickens in the OFM group exhibited persistent diarrhea symptoms, and fecal pH was significantly decreased ($P<0.05$) while fecal moisture content was significantly increased ($P<0.05$) at 14 and 21 days of age; 3) For villus height, crypt depth, and villus height/crypt depth in the ileum of 21-day-old broiler chickens, there were no significant differences among the three groups ($P>0.05$); 4) The contents of glutathione, oxidized glutathione, lipid peroxides, and malondialdehyde, as well as the glutathione/oxidized glutathione ratio in the crop of broiler chickens showed no significant differences among the three groups ($P>0.05$), whereas the OFM group significantly increased the contents of lipid peroxides and malondialdehyde in the ileum of broiler chickens compared with the CON and FM groups ($P<0.05$). These results suggest that oxidized fish meal had no significant effect on ileal morphological structure in broiler chickens, but significantly disrupted the redox balance status in the ileum, caused diarrhea, affected gastrointestinal tract function, and significantly reduced growth performance in early-stage broiler chickens.

Full Text

Effects of Oxidized Fish Meal on Growth Performance, Digestive Tract Structure and Function of Broiler Chickens

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Abstract: This experiment was conducted to investigate the effects of oxidized fish meal protein on the growth performance, digestive tract structure, and function of broiler chickens. A total of 180 healthy 1-day-old male broilers with similar body weight were randomly allocated into 3 groups with 6 replicates of 10 birds each. The three groups were fed a control diet (CON group), a diet containing 2% normal fish meal (FM group), and a diet containing 2% oxidized fish meal (OFM group) for a 21-day experimental period. The results showed that: (1) Compared with the CON and FM groups, the OFM group significantly decreased body weight and average daily gain ($P<0.05$) while significantly increasing the feed-to-gain ratio ($P<0.05$). (2) The OFM group caused persistent diarrhea symptoms, significantly reduced fecal pH, and significantly increased fecal moisture content in 14- and 21-day-old broilers ($P<0.05$). (3) No significant differences were observed among the three groups in ileal villus height, crypt depth, or villus height-to-crypt depth ratio in 21-day-old broilers ($P>0.05$). (4) No significant differences were found among groups in crop glutathione, oxidized glutathione, lipid peroxide, malondialdehyde content, or glutathione-to-oxidized glutathione ratio ($P>0.05$), but the OFM group significantly increased ileal lipid peroxide and malondialdehyde content compared with the CON and FM groups ($P<0.05$). These results indicate that oxidized fish meal did not significantly affect ileal morphology but substantially disrupted the redox balance in the ileum, induced diarrhea, impaired digestive function, and significantly reduced growth performance in early-stage broiler chickens.

Keywords: broiler chickens; oxidized fish meal; digestive tract; growth performance

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Introduction

Antioxidant and pro-oxidant reactions occurring during fish meal processing and storage significantly affect its quality. While high temperatures from lipid oxidation in fish meal have toxic effects on protein quality, research on the direct impact of lipid oxidation on fish meal protein quality remains limited. Lipid peroxides (LPO), such as hydroxynonenal, malondialdehyde (MDA), and acrolein, can react with lysine, histidine, and cysteine residues, and more importantly,

oxidation is a chain reaction whose free radicals may directly attack proteins. Protein oxidation is defined as covalent modification of proteins induced directly by free radicals or by secondary products of oxidation reactions.

Fish meal is rich in unsaturated fatty acids and prone to oxidative rancidity, which accelerates protein deterioration. Studies have shown that when low-fat and high-fat fish are heated at 40–115 °C for 20 minutes, free sulfhydryl (-SH) content in proteins decreases linearly while disulfide bond (S–S) content increases. Even during low-temperature storage (below 0 °C), fish meal exhibits reduced sulfhydryl content. However, conversion of sulfhydryl groups to disulfide bonds requires temperatures above 50 °C, with rapid reaction occurring at 95 °C and reaching equilibrium after 20 minutes. Conversion of internal protein sulfhydryl groups to disulfide bonds creates cross-linked, insoluble protein colloidal structures, and changes in protein biochemical structure (free sulfhydryl content, sulfhydryl/(sulfhydryl+disulfide) ratio) can alter dietary protein digestibility. Research has reported that oxidized soy protein generates free radicals that reduce antioxidant status and cause redox imbalance in the digestive tract of rats, while other studies found that oxidized soy protein decreased the activity of multiple enzymes in the foregut of broiler chickens. Therefore, we hypothesized that oxidized dietary protein may induce functional changes in the digestive tract of broiler chickens and affect growth performance. Since fish meal is rich in unsaturated fatty acids and susceptible to oxidative rancidity during processing and storage, accelerating protein deterioration, and since fish oil may induce protein oxidation and covalent modification, this study aimed to investigate the effects of oxidized fish meal on growth performance, digestive tract structure, and function in broiler chickens to elucidate the underlying mechanisms of its adverse effects and provide a theoretical basis for mitigating these effects in practice.

Materials and Methods

1.1 Experimental Materials

Normal domestic fish meal served as the basal material. Following the method reported by Lagrain et al., oxidized fish meal was prepared by mixing 20 g of fish meal with 100 mL of distilled water containing 8 mL/L hydrogen peroxide, heating at 95 °C for 1 hour, then air-drying and grinding. The normal and oxidized fish meals used in the experiment were of the same type.

1.2 Experimental Design

A total of 180 healthy 1-day-old Arbor Acres male broilers (provided by Beijing Huadu Broiler Company) with similar body weight [(37.47±0.77) g] were used in a single-factor completely randomized design. The broilers were divided into 3 groups with 6 replicates of 10 birds each. The control (CON) group, fish meal (FM) group, and oxidized fish meal (OFM) group were fed a control diet, a diet containing 2% normal fish meal, and a diet containing 2% oxidized fish meal,

respectively. Dietary nutrient levels followed Aviagen' s breed recommendation standards, with diet composition and nutrient levels shown in Table 1 .

1.3 Husbandry Management

The 21-day experimental period allowed all broilers ad libitum access to feed and water. Birds were housed in battery cages with a lighting schedule of 1 hour dark/23 hours light. Temperature was artificially controlled at approximately 33 °C for the first 4 days, then reduced by 2 °C weekly until reaching 22 °C. Vaccination with combined Newcastle disease and infectious bronchitis vaccine was performed at 7 days of age (eye and nose drops), and infectious bursal disease vaccine was administered at 14 days of age (drinking water). Feed consumption and health status were observed and recorded daily.

1.4 Measurements

1.4.1 Free Sulfhydryl Content in Oxidized Fish Meal Free sulfhydryl content was determined using Ellman' s method, following procedures reported by Beveridge et al. and Buttkus.

1.4.2 Growth Performance Bird health status was recorded daily during the experiment. At 07:00 on day 21, fasting body weight was measured per replicate. Based on experimental records, body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated per replicate.

1.4.3 Fecal pH and Moisture Content Fresh feces were collected at 14 and 21 days of age, mixed, and pH (Testo 206 portable pH meter) and moisture content were measured.

1.4.4 Ileum Morphology and Oxidative Status At 21 days of age, one bird per replicate was selected based on average body weight, euthanized by electric shock, and the abdominal cavity was opened to collect two 2-cm ileal segments and 3 cm^2 of crop tissue. One ileal segment was rinsed with physiological saline, fixed in 4% formaldehyde solution, and processed for paraffin sections. Fixed tissues were trimmed, dehydrated, embedded, sectioned, and stained with hematoxylin-eosin (HE). Villus height and crypt depth were measured at 100× magnification using a micrometer, and villus height-to-crypt depth ratio (V/C) was calculated.

Crop and the other ileal segment were weighed and ground to prepare tissue homogenates. Total protein in tissues was measured using a UV spectrophotometer (Thermo Fisher Scientific, Nano Drop 2000) for biochemical analysis. Glutathione (GSH) and oxidized glutathione (GSSG) contents were determined using Nanjing Jiancheng Bioengineering Institute kits A061 and A061-1, following the method of Tietze, with reduced glutathione calculated as the difference

between the two. MDA content was measured using kit A003-1 with the thiobarbituric acid (TBA) method, following Todorova et al. LPO content was also determined by the TBA method using kit A106.

1.5 Statistical Analysis

All experimental data were organized using Excel 2007 and analyzed by one-way ANOVA using SPSS 19.0 software. Differences among group means were tested for significance using LSD multiple comparison. Data were expressed as mean ± standard error, with $P<0.05$ considered statistically significant.

Results

2.1 Free Sulfhydryl Content in Fish Meal

The free sulfhydryl content in normal fish meal was 31.11 mol/g, while that in oxidized fish meal processed with 8 mL/L hydrogen peroxide was 13.32 mol/g, representing a 57.18% reduction.

2.2 Effects of Oxidized Fish Meal on Growth Performance of Broilers

As shown in Table 2 , for 1–21 day-old broilers, no significant differences were observed in ADFI among the three groups ($P>0.05$). Body weight, ADG, and F/G did not differ significantly between the CON and FM groups ($P>0.05$). However, compared with the CON and FM groups, the OFM group significantly decreased body weight and ADG ($P<0.05$) while significantly increasing F/G ($P<0.05$).

2.3 Effects of Oxidized Fish Meal on Fecal pH and Moisture Content of Broilers

As presented in Table 3 , the OFM group significantly decreased fecal pH and increased fecal moisture content compared with the CON and FM groups ($P<0.05$). After feeding the oxidized fish meal diet, fecal moisture content in 14- and 21-day-old broilers reached 77.88% and 75.93%, respectively.

2.4 Effects of Oxidized Fish Meal on Ileum Morphology of Broilers

As shown in Table 4 , no significant differences were observed among the three groups in ileal villus height, crypt depth, or V/C ratio in 21-day-old broilers ($P>0.05$).

2.5 Effects of Oxidized Fish Meal on Oxidative Status of Crop and Ileum

As shown in Table 5 , the OFM group did not significantly affect GSH, GSSG, GSH/GSSG ratio, or LPO and MDA contents in the crop of 21-day-old broilers compared with the CON and FM groups ($P>0.05$). However, feeding the normal

fish meal diet significantly increased ileal GSH concentration compared with the other two diets ($P<0.05$). The OFM group significantly increased ileal LPO and MDA contents compared with the CON and FM groups ($P<0.05$).

Discussion

3.1 Free Sulfhydryl Content in Oxidized Fish Meal

The conversion of free sulfhydryl groups to disulfide bonds is a significant change during protein oxidation and serves as an indicator for detecting protein oxidation. Ellman' s method is widely used for measuring free sulfhydryl content in proteins, which is why it was employed in this study. Research indicates that even during low-temperature storage (below 0 °C), fish meal exhibits reduced sulfhydryl content. When low-fat and high-fat fish are heated at 40–115 °C for 20 minutes, free sulfhydryl content in proteins decreases linearly while disulfide bond content increases. Free sulfhydryl content can be used to evaluate feed or ingredient quality, with high-quality feed proteins reportedly containing significantly higher free sulfhydryl content than low-quality alternatives. Studies have reported disulfide bond content in fish meal diets ranging from 23.3–32.7 nmol/mg prot, while initial fish meal sulfhydryl content was 16.5–17.3 mol/g, decreasing to 12.9–14.9 mol/g after 12 days of low-temperature storage. In our study, normal fish meal contained 31.11 mol/g sulfhydryl groups, which decreased to 13.32 mol/g after oxidation. Differences from previous studies may be attributed to variations in fish species used for meal production. Sulfhydryl oxidation is just one type of amino acid side chain oxidation; protein oxidation may also cause protein backbone oxidation or fragmentation.

3.2 Effects of Oxidized Fish Meal on Growth Performance of Broilers

Protein oxidation induces a series of structural modifications that reduce protein quality and digestibility, causing loss of nutritional value and reduced bioavailability—factors that contribute to decreased growth performance in broilers. Studies have found that moderately rancid fish meal significantly reduced final body weight, daily gain, and growth rate in black tiger shrimp compared with highly rancid fish meal, suggesting that protein oxidation caused by lipid peroxidation, rather than lipid peroxidation itself, may be the critical factor affecting growth performance. In our study, feeding oxidized fish meal significantly decreased body weight and ADG while increasing F/G in broilers, consistent with findings that heat-treated soy protein isolate with reduced sulfhydryl content significantly decreased average body weight in broilers.

3.3 Effects of Oxidized Fish Meal on Fecal pH and Moisture Content of Broilers

Medical research indicates that fecal appearance and consistency represent important diagnostic clues for disease. Based on visual scoring criteria, oxidized fish meal produced loose feces with low conical formation; fecal moisture content

exceeded 75%, and although no free water was observed, broilers exhibited persistent diarrhea symptoms. Studies using oxidized vegetable oil or oxidized fish oil in broilers and weaned piglets did not report diarrhea symptoms, suggesting that oxidized fish meal specifically induces diarrhea in broilers. Impaired intestinal integrity can reduce water reabsorption, further causing diarrhea.

In this study, the OFM group significantly reduced fecal pH. Microbial metabolites (lactic acid, acetic acid, propionic acid, and butyric acid) are primary contributors to reduced intestinal mucosal and fecal pH. While moderately acidic feces are considered normal, excessive acidity may indicate digestive tract pathology. Research in weaned piglets has associated fecal pH with diarrhea, often using reduced fecal pH as a diagnostic indicator for osmotic diarrhea. Although similar reports are lacking in poultry, the combined results of fecal appearance, moisture content, and pH demonstrate that oxidized fish meal impairs digestive function in broilers.

3.4 Effects of Oxidized Fish Meal on Ileum Morphology of Broilers

Numerous studies have used dietary effects on intestinal morphology (villus height, crypt depth, V/C ratio) as indicators of animal health, with some linking local or systemic inflammatory responses to small intestinal villus height. Although 2% oxidized fish meal significantly affected early-stage broiler growth performance, ileal morphology was not affected. Previous research has suggested that protein oxidation damages digestive tract structure, with heat-oxidized soy protein significantly reducing relative weights of the foregut and ileum and potentially negatively affecting digestive organ development. Other studies have reported that advanced protein oxidation products induce intestinal tissue damage in inflammatory bowel disease patients.

3.5 Effects of Oxidized Fish Meal on Oxidative Status of Crop and Ileum

The digestive tract includes the gastrointestinal tract, comprising anterior digestive organs and posterior intestinal segments. The crop is an important site of microbial activity in broilers, with approximately half of ingested food remaining in the crop 3 hours after feeding. Therefore, in addition to the ileum, this study examined crop effects, finding that oxidized fish meal did not significantly affect crop oxidative status but significantly increased LPO and MDA contents in the ileum of early-stage broilers.

Antioxidant capacity in the digestive tract plays a crucial role in maintaining normal digestive function. Our results indicate that oxidized fish meal induced a stress state that disrupted redox balance in the digestive tract, consistent with previous studies using oxidized feed ingredients. Oxidized soy protein disrupted oxidative balance and reduced antioxidant capacity in rat digestive tracts, while oxidized fish oil induced oxidative stress in weaned piglets, reducing antioxidant enzyme activities and increasing MDA content. Gluten protein anti-

gen fragments entering intestinal epithelial cells can activate nuclear factor- B (NF- B), inducing transcription of pro-inflammatory cytokines, cyclooxygenase 2 (COX2), and inducible nitric oxide synthase (iNOS), leading to massive production of prostaglandins and nitric oxide metabolites and ultimately causing epithelial cell oxidative stress. Advanced protein oxidation products have also been reported to induce human intestinal epithelial cell death through oxidation-mediated pathways. Combined with ileal morphology results, 2% oxidized fish meal only altered redox balance in the ileum without causing ileal epithelial cell damage.

Conclusion

Oxidized fish meal did not significantly affect ileal morphology but substantially disrupted ileal redox balance, induced diarrhea, impaired digestive function, and significantly reduced growth performance in early-stage broiler chickens.

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