# Effects of Eupolyphaga sinensis Peptide on Growth Performance, Meat Quality, Organ Indices, and Antioxidant Capacity of Broiler Chickens under Oxidative Stress (Postprint)

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## Abstract

This experiment aimed to investigate the effects of ground beetle peptide on growth performance, meat quality, organ indices, and antioxidant capacity of broiler chickens under hydrocortisone-induced oxidative stress. A total of 180 one-day-old healthy Arbor Acres (AA) broiler chickens were randomly divided into 5 groups: a control group, a model group (25 mg/L hydrocortisone), and three ground beetle peptide groups [0.4, 0.8, and 1.6 g/kg BW], with 6 replicates per group and 6 chickens per replicate. From day 4, ground beetle peptide was added to the drinking water of the three ground beetle peptide groups until day 21; from day 8, hydrocortisone was added to the drinking water of all groups except the control group for 5 consecutive days. The experimental period was 21 days. The results showed: 1) At 14 days of age, the serum corticosterone content of broiler chickens in the model group was significantly higher than that in the control group and the ground beetle peptide groups ($P<0.05$). 2) During days 1-14, compared with the control group, the average daily gain (ADG) of broiler chickens in the model group was significantly decreased ($P<0.05$), and the feed-to-gain ratio (F/G) was significantly increased ($P<0.05$); compared with the model group, the F/G of the ground beetle peptide groups was significantly decreased ($P<0.05$). During days 15-21, the ADG and average daily feed intake (ADFI) of the model group were significantly lower than those of the control group ($P<0.05$). During days 1-21, the ADG and ADFI of the model group were significantly lower than those of the control group ($P<0.05$), and the F/G was significantly higher than that of the control group ($P<0.05$); compared with the model group, the F/G of the 0.4 and 1.6 g/kg BW ground beetle peptide groups was significantly decreased ($P<0.05$). 3) At 14 days of age, compared with the control group, the breast muscle pH at 45 min and redness (a*) value of broiler chickens in the model group were significantly decreased (P<0.05), and*

*the drip loss of breast and thigh muscles was significantly increased (P<0.05); compared with the model group, the breast muscle pH at 45 min of the 0.8 g/kg BW ground beetle peptide group was significantly increased (P<0.05), the breast muscle drip loss was significantly decreased (P<0.05), and the thigh muscle drip loss of the 0.4 and 1.6 g/kg BW ground beetle peptide groups was significantly decreased (P<0.05). At 21 days of age, compared with the model group, the breast muscle a* value of the 0.4 g/kg BW ground beetle peptide group was significantly increased (P<0.05), and the breast muscle drip loss was significantly decreased (P<0.05). 4) At 14 days of age, the spleen, thymus, and bursa of Fabricius indices of broiler chickens in the model group were significantly lower than those of the control group (P<0.05); at 21 days of age, the bursa of Fabricius index of the model group was significantly lower than that of the control group and the 1.6 g/kg BW ground beetle peptide group (P<0.05). 5) At 14 days of age, the superoxide dismutase (SOD) activity in liver and breast muscle of broiler chickens in the model group was significantly lower than that of the control group (P<0.05), the glutathione peroxidase (GSH-Px) activity in liver and breast muscle was significantly lower than that of the control group and the 0.4 g/kg BW ground beetle peptide group (P<0.05), and the catalase (CAT) activity in liver and breast muscle was significantly lower than that of the control group and the 0.8 and 1.6 g/kg BW ground beetle peptide groups (P<0.05). At both 14 and 21 days of age, the malondialdehyde (MDA) content in liver and breast muscle of the model group was significantly higher than that of the control group (P<0.05). These results suggest that ground beetle peptide can effectively alleviate stress response, promote growth, improve meat quality, and enhance antioxidant capacity of broiler chickens under oxidative stress.

## Full Text

# Effects of Eupolyphaga sinensis Walker Polypeptides on Growth Performance, Meat Quality, Organ Indexes and Antioxidant Capacity of Broilers Under Oxidative Stress

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**Abstract:** This experiment investigated the effects of Eupolyphaga sinensis Walker polypeptides (ESWPs) on growth performance, meat quality, organ indexes, and antioxidant capacity in broiler chickens under oxidative stress induced by hydrocortisone. One hundred eighty 1-day-old healthy Arbor Acres (AA) broilers were randomly allocated into five groups: a control group, a model group (25 mg/L hydrocortisone), and three ESWPs groups (0.4, 0.8, and 1.6 g/kg body weight). Each group comprised six replicates with six broilers per replicate. From day 4 to day 21, ESWPs were administered via drinking water to the three treatment groups. From day 8, hydrocortisone was added to

the drinking water of all groups except the control group for five consecutive days. The experimental period lasted 21 days. The results showed: (1) At 14 days of age, serum corticosterone content in the model group was significantly higher than in the control and ESWPs groups (P<0.05). (2) During days 1–14, compared with the control group, the model group exhibited significantly reduced average daily gain (ADG) and increased feed-to-gain ratio (F/G) (P<0.05); compared with the model group, ESWPs groups showed significantly decreased F/G (P<0.05). During days 15–21, ADG and average daily feed intake (ADFI) in the model group were significantly lower than in the control group (P<0.05). Over the entire 1–21 day period, ADG and ADFI in the model group were significantly lower than in the control group (P<0.05), while F/G was significantly higher (P<0.05); the 0.4 and 1.6 g/kg BW ESWPs groups showed significantly lower F/G compared with the model group (P<0.05). (3) At 14 days of age, compared with the control group, the model group exhibited significantly decreased breast muscle pH at 45 min post-mortem (pH ) and redness (a*) values (P<0.05), along with significantly increased drip loss in both breast and leg muscles (P<0.05); compared with the model group, the 0.8 g/kg BW ESWPs group showed significantly increased breast muscle pH and decreased breast muscle drip loss (P<0.05), while the 0.4 and 1.6 g/kg BW ESWPs groups demonstrated significantly reduced leg muscle drip loss (P<0.05). At 21 days of age, the 0.4 g/kg BW ESWPs group exhibited significantly increased breast muscle a* values and decreased breast muscle drip loss compared with the model group (P<0.05). (4) At 14 days of age, the spleen, thymus, and bursa of Fabricius indexes in the model group were significantly lower than in the control group (P<0.05). At 21 days of age, the bursa of Fabricius index in the model group was significantly lower than in both the control group and the 1.6 g/kg BW ESWPs group (P<0.05). (5) At 14 days of age, hepatic and breast muscle superoxide dismutase (SOD) activity in the model group was significantly lower than in the control group (P<0.05); hepatic and breast muscle glutathione peroxidase (GSH-Px) activity was significantly lower than in the control and 0.4 g/kg BW ESWPs groups (P<0.05); hepatic and breast muscle catalase (CAT) activity was significantly lower than in the control and 0.8 and 1.6 g/kg BW ESWPs groups (P<0.05). At both 14 and 21 days of age, hepatic and breast muscle malondialdehyde (MDA) content in the model group was significantly higher than in the control group (P<0.05). These results indicate that ESWPs can effectively alleviate stress responses, promote growth, improve meat quality, and enhance antioxidant capacity in broilers under oxidative stress.

**Keywords:** Eupolyphaga sinensis Walker polypeptides; broilers; oxidative stress; meat quality; antioxidant capacity

## Introduction

Oxidative stress refers to a pathological process in which excessive free radicals overwhelm the body' s scavenging capacity following endogenous or exoge-

nous stimulation, disrupting redox homeostasis and causing oxidative damage [1]. In modern intensive production systems, environmental, nutritional, and transportation factors frequently induce oxidative stress, compromising animal performance, immune function, and product quality. Consequently, developing safe and effective natural antioxidants represents a critical research priority.

Recent studies have identified antioxidant peptides derived from enzymatic hydrolysis of various plant and animal proteins. These peptides scavenge excess oxygen free radicals, inhibit lipid peroxidation, and exhibit high bioavailability [2]. Bioactive peptides demonstrate beneficial effects in immune modulation, cholesterol reduction, antimicrobial activity, and antioxidant defense [3]. For instance, Achyranthes bidentata polypeptides significantly attenuate oxidative stress and protect myocardial function in rats with ischemia-reperfusion injury [4], while rice protein peptides ameliorate heat stress-induced performance decline and metabolic disturbances in laying hens [5].

Eupolyphaga sinensis Walker (ESW) exhibits pharmacological properties including lipid regulation, anti-tumor activity, immunomodulation, and antioxidant effects [6]. Although Eupolyphaga sinensis Walker polypeptides (ESWPs) have demonstrated antioxidant activity, existing research remains limited to basic extraction, activity analysis, and murine models. No studies have investigated the effects of ESWPs on poultry under oxidative stress conditions.

This study established an oxidative stress model in broilers via hydrocortisone administration in drinking water to evaluate the effects of different ESWPs levels on growth performance, meat quality, organ indexes, and antioxidant capacity, thereby providing a theoretical basis for ESWPs application in broiler production.

## Materials and Methods

### Experimental Materials

Hydrocortisone (white powder, purity  99%, solubility 100 mg/mL) was purchased from Shanxi Kelong Veterinary Medicine Co., Ltd. ESWPs were prepared in our laboratory using protease hydrolysis of ESW powder. Tricine-SDS-PAGE analysis determined the molecular weight of ESWPs as 8–9 kDa, and biuret assay measured the concentration at 45 mg/mL.

### Experimental Design and Diets

One hundred eighty 1-day-old healthy Arbor Acres (AA) male broilers were randomly assigned to five groups: control, model (25 mg/L hydrocortisone), and three ESWPs groups (0.4, 0.8, and 1.6 g/kg BW). Each group contained six replicates of six broilers. ESWPs were administered via drinking water from day 4 to day 21. Hydrocortisone was added to drinking water of all groups except the control from day 8 for five consecutive days. The experiment lasted 21 days with ad libitum access to feed and water. Clinical signs and mortality

were monitored daily. The basal diet was formulated according to NRC (1994) and Chinese Feeding Standards (NY/T 33–2004) as a corn-soybean meal powder diet. Composition and nutrient levels are presented in .

### Sample Collection and Preparation

On days 14 and 21, 18 broilers per group were weighed (after 12-hour fasting) and blood samples were collected via cardiac puncture. Serum was harvested by centrifugation and stored at -20°C. Following euthanasia, left breast and leg muscles were excised and weighed; portions were immediately analyzed for meat quality, with remainder stored at -20°C along with liver samples. Pancreas, thymus, spleen, and bursa of Fabricius were weighed for organ index calculation. One-gram tissue samples (liver, breast muscle) were homogenized in 9 mL ice-cold saline to prepare 10% homogenates, centrifuged at 3,500 r/min for 15 min, and supernatants stored at -20°C.

### Measurements

**Growth Performance:** Body weight and feed consumption were recorded on days 1, 14, and 21 per replicate to calculate ADG (g/d), ADFI (g/d), and F/G.

**Organ Indexes:** Calculated as: Organ index (mg/g) = organ weight (mg) / live body weight (g).

**Meat Quality:** pH was measured at 45 min and 24 h post-mortem on 2-g muscle samples homogenized in 10 volumes of ultrapure water. Color parameters (lightness L*, redness a*, yellowness b*) were measured using a colorimeter on standardized muscle locations. Drip loss was determined on 35 cm × 15 cm × 10 mm muscle blocks weighed before (W ) and after (W ) suspension in inflated plastic bags at 4°C for 24 h: Drip loss (%) = (W -W )/W × 100.

**Serum Corticosterone:** Determined using commercial assay kits according to manufacturer instructions.

**Antioxidant Enzyme Activities and Peroxide Content:** Hepatic and breast muscle SOD, CAT, GSH-Px activities and MDA content were measured using commercial kits.

### Statistical Analysis

Data were analyzed using GraphPad Prism 6.0 software via one-way ANOVA with LSD post-hoc tests. Results are expressed as mean ± SD. Statistical significance was declared at $P<0.05$.

## Results

### Effects of ESWPs on Corticosterone Secretion in Oxidative Stress

As shown in , serum corticosterone content at 14 days of age was significantly higher in the model group than in the control and all ESWPs groups (P<0.05). Although ESWPs groups showed numerically increased corticosterone compared with the control group, differences were not significant (P>0.05), with the 1.6 g/kg BW group exhibiting the lowest value. At 21 days of age, the model group maintained elevated corticosterone levels, though differences among groups were not statistically significant (P>0.05).

### Effects of ESWPs on Growth Performance Under Oxidative Stress

presents the growth performance data. During days 1–14, the model group exhibited significantly reduced ADG and increased F/G compared with the control group (P<0.05), while ADFI showed a decreasing trend (P>0.05). ESWPs supplementation significantly decreased F/G compared with the model group (P<0.05), with numerical improvements in ADG and reduced ADFI. During days 15–21, ADG and ADFI in the model group were significantly lower than in the control group (P<0.05), while ESWPs groups showed numerical improvements without significant differences. Over the entire 21-day period, the model group demonstrated significantly lower ADG and ADFI and higher F/G than the control group (P<0.05). The 0.4 and 1.6 g/kg BW ESWPs groups significantly reduced F/G compared with the model group (P<0.05).

### Effects of ESWPs on Meat Quality Under Oxidative Stress

Breast muscle quality data are summarized in . At 14 days of age, no significant differences were observed in relative breast muscle weight among groups (P>0.05). The model group exhibited significantly lower pH and a* values compared with the control group (P<0.05), while L* and b* values showed increasing trends (P>0.05). Breast muscle drip loss was significantly higher in the model group than in the control group (P<0.05). ESWPs supplementation reduced drip loss, with the 0.8 g/kg BW group showing significant reduction (P<0.05). At 21 days of age, the 0.4 g/kg BW ESWPs group demonstrated significantly higher a* values and lower drip loss compared with the model group (P<0.05), while other parameters showed no significant differences.

Leg muscle quality results are presented in . At 14 days of age, relative leg muscle weight did not differ among groups (P>0.05). The model group exhibited significantly higher leg muscle drip loss than the control group (P<0.05), while ESWPs groups showed reduced values, with significant reductions in the 0.4 and 1.6 g/kg BW groups (P<0.05). At 21 days of age, no significant differences were detected in any leg muscle quality parameters among groups (P>0.05).

### Effects of ESWPs on Organ Indexes Under Oxidative Stress

shows the organ index results. At 14 days of age, pancreatic indexes were similar across groups (P>0.05). The model group exhibited significantly lower spleen, thymus, and bursa of Fabricius indexes compared with the control group (P<0.05). ESWPs supplementation improved these indexes, with the 0.4 and 0.8 g/kg BW groups showing significantly higher spleen indexes, the 1.6 g/kg BW group showing improved thymus index, and the 0.4 g/kg BW group demonstrating significantly higher bursa of Fabricius index compared with the model group (P<0.05). At 21 days of age, pancreatic, splenic, and thymic indexes showed similar trends without significant differences (P>0.05). The model group maintained significantly lower bursa of Fabricius index than the control group (P<0.05), while the 1.6 g/kg BW ESWPs group showed significant improvement over the model group (P<0.05).

### Effects of ESWPs on Hepatic Antioxidant Capacity Under Oxidative Stress

As shown in , at 14 days of age, hepatic SOD activity in the model group was significantly lower than in the control group (P<0.05), while the 1.6 g/kg BW ESWPs group exhibited significantly higher activity than the model group (P<0.05). Hepatic GSH-Px activity in the model group was significantly lower than in the control and 0.4, 0.8 g/kg BW ESWPs groups (P<0.05). Hepatic CAT activity in the control and 0.8, 1.6 g/kg BW ESWPs groups was significantly higher than in the model group (P<0.05). Hepatic MDA content in the model group was significantly elevated compared with the control group (P<0.05), while the 0.8 and 1.6 g/kg BW ESWPs groups showed significant reductions (P<0.05). At 21 days of age, hepatic antioxidant enzyme activities in the model group were numerically lower than in the control and ESWPs groups without significant differences (P>0.05), while hepatic MDA content in the control and 1.6 g/kg BW ESWPs groups remained significantly lower than in the model group (P<0.05).

### Effects of ESWPs on Breast Muscle Antioxidant Capacity Under Oxidative Stress

presents the breast muscle antioxidant capacity data. At 14 days of age, breast muscle SOD activity in the model group was significantly lower than in the control and 0.4 g/kg BW ESWPs groups (P<0.05). Breast muscle GSH-Px activity in the model group was significantly reduced compared with the control and 0.4, 1.6 g/kg BW ESWPs groups (P<0.05). Breast muscle CAT activity in the model group was significantly lower than in all other groups (P<0.05). Breast muscle MDA content in the model group was significantly higher than in the control group (P<0.05), while the 0.4 g/kg BW ESWPs group showed significant reduction (P<0.05). At 21 days of age, no significant differences were observed in SOD or GSH-Px activities among groups (P>0.05). Breast muscle CAT activity in the model group was significantly lower than in the 0.8 g/kg

BW ESWPs group (P<0.05). Breast muscle MDA content in the model group remained significantly elevated compared with the control group (P<0.05), while ESWPs groups showed numerical reductions.

## Discussion

### Establishment of the Hydrocortisone-Induced Oxidative Stress Model

Oxidative stress represents a ubiquitous challenge in intensive livestock production, causing substantial economic losses. During oxidative stress, excessive intracellular free radicals attack polyunsaturated fatty acids in cell membranes, compromising membrane structure and function, oxidizing proteins and nucleic acids, altering enzyme activities, impairing tissue function, reducing disease resistance, and predisposing animals to various diseases [7]. Since the discovery of potential toxicity and carcinogenicity of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) in 1980, developing natural antioxidants has become increasingly important.

Under oxidative stress, the hypothalamus releases corticotropin-releasing hormone (CRH), stimulating anterior pituitary secretion of adrenocorticotropic hormone (ACTH), which subsequently promotes glucocorticoid release from the adrenal cortex [8]. Corticosterone, a key metabolic regulator, critically influences carbohydrate, lipid, and protein metabolism. Elevated blood corticosterone reflects the physiological response to stress. Previous studies demonstrated that immune challenge and lipopolysaccharide (LPS) stimulation significantly increased plasma corticosterone in chicks, with vitamin E supplementation providing effective mitigation [9]. Similarly, Newcastle disease vaccination-induced immune stress significantly elevated serum ACTH and corticosterone concentrations along with hypothalamic CRH mRNA expression [10]. Our findings confirm that hydrocortisone administration in drinking water significantly increased serum corticosterone, successfully inducing oxidative stress in broilers.

### Effects of ESWPs on Growth Performance Under Oxidative Stress

Dexamethasone-induced oxidative stress has been shown to significantly reduce ADFI and ADG, inhibiting broiler growth [11-12]. Dietary oxidized soybean oil progressively decreased feed conversion efficiency and egg production in laying hens [13], consistent with findings by Eid et al. [14]. Our results demonstrate that the oxidative stress model group exhibited reduced ADG and ADFI with increased F/G compared with the control group. ESWPs supplementation improved ADG and ADFI while reducing F/G, indicating that ESWPs effectively mitigate the detrimental effects of oxidative stress on broiler growth performance.

### Effects of ESWPs on Meat Quality Under Oxidative Stress

Stress reduces protein deposition and muscle weight in broilers, decreases muscle yield, promotes fat synthesis, increases abdominal fat percentage, and reduces carcass edible portions [15]. Oxidative stress accelerates glycolysis through reactive oxygen species (ROS)-mediated oxidation of lipids and proteins, causing rapid pH decline, increased drip loss, protein denaturation, water exudation, and pale muscle color [16-18]. Our findings show that oxidative stress decreased muscle pH, increased L* values, reduced a* values, and elevated drip loss, while ESWPs supplementation effectively ameliorated these adverse effects on meat quality.

### Effects of ESWPs on Organ Indexes Under Oxidative Stress

Oxidative stress exerts widespread effects, causing varying degrees of oxidative damage to muscles and major organs. Organ indexes serve as crucial in vivo indicators for assessing drug toxicity and immunomodulatory effects. Limited information exists regarding ESWPs effects on broiler organ indexes. Our results demonstrate that oxidative stress reduced organ indexes compared with the control group, while ESWPs supplementation improved these indexes and enhanced organ development, suggesting that ESWPs promote growth and ameliorate oxidative damage in broilers.

### Effects of ESWPs on Hepatic and Breast Muscle Antioxidant Capacity Under Oxidative Stress

Oxidative stress severely disrupts the balance between pro-oxidant and antioxidant systems in broilers [19-20]. Excessive MDA accumulation inhibits antioxidant enzyme activities and exacerbates DNA and protein oxidative damage, with stress increasing MDA content 2–4 fold in breast and skeletal muscles [21]. Dexamethasone-induced oxidative stress significantly decreased serum GSH-Px activity and total antioxidant capacity (T-AOC) [22], while diquat-induced oxidative stress in piglets reduced blood SOD, GSH-Px, and CAT activities with increased MDA content [23]. Our evaluation of antioxidant enzyme activities and MDA content in liver and breast muscle confirmed successful induction of oxidative stress by hydrocortisone, causing oxidative damage. ESWPs supplementation increased antioxidant enzyme activities and reduced MDA content, demonstrating that ESWPs effectively alleviate hydrocortisone-induced oxidative stress and enhance antioxidant capacity in broiler liver and breast muscle.

## Conclusion

ESWPs alleviate hydrocortisone-induced oxidative damage by enhancing hepatic and muscular antioxidant enzyme activities and reducing lipid peroxidation, thereby improving organ development, meat quality, and post-damage recovery in broilers. Under our experimental conditions, the optimal ESWPs supplementation level in drinking water ranged from 0.4 to 0.8 g/kg BW.

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