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Effects of Soy Isoflavone on Performance, Meat Quality and Antioxidative Property of Male Broilers Fed Oxidized Fish Oil*

S. Q. Jiang[1, 2], Z. Y. Jiang[2, **], Y. C. Lin[2], P. B. Xi[2] and X. Y. Ma[2]

[1]College of Animal Science, Zhejiang University, Hangzhou 310029, P. R. China

ABSTRACT : This study was conducted to determine the effect of dietary soy isoflavone (ISF, 0 and 20 mg/kg) on performance, meat quality and antioxidative property in male broilers. Six replicates of 45 birds (42 d old) were used for each treatment. The birds were fed soybean meal-free diets containing 3% oxidized fish oil (acid value, 6.76 mg potassium hydroxide/g; and peroxide value, 6.18 meq/kg) for 3 wk. The results showed that average daily gain, feed intake, feed conversion and carcass traits were not affected by soy ISF. Compared with the control group, breast muscle color redness value and water holding capacity were increased ($p<0.05$) by ISF supplementation. The activity of plasma catalase was increased by supplementing with 20 mg ISF/kg diet. In breast muscle, Broilers fed 20 mg ISF/kg had decreased production of malondialdehyde and lactic acid. The ISF supplementation elevated total antioxidative capacity and activities of total superoxide dismutase and glutathione peroxidase. The results indicated that dietary ISF could increase redness and water holding capacity of meat, and antioxidative property of meat in male broilers fed oxidized fish oil diet. (**Key Words :** Isoflavone, Oxidized Fish Oil, Broiler, Meat Quality, Antioxidation)

INTRODUCTION

Poultry diets are often supplemented with fish oils that contains a high amount of C20:5 n-3 (eicosapentaenoic acid, EPA) and C22:6 n-3 (docosahexaenoic acid, DHA). Fish oil addition is more efficient than vegetable fat sources in producing long-chain n-3 polyunsaturated fatty acids (PUFA) enrichment in meat. However, a higher PUFA content of poultry meat increases the degree of unsaturation and, as a result, also increases the susceptibility to oxidation. This may then lead to off-flavors and off-odors and, consequently, lower consumer acceptability. The use of fish oils at concentrations above 2% in poultry diets may entail several sensory problems that compromise meat quality (Hargis and van Elswyk, 1993). Great concern is being given for improving meat quality with antioxidant additives in broiler chickens. Sheldon et al. (1997) reported that dietary vitamin E levels significantly influenced the oxidative stability and functionality of turkey breast meat. Plants, such as green tea (Tang et al., 2000; Yang et al., 2003), rosemary and sage (Lopez et al., 1998) that contain high concentrations of antioxidants have been demonstrated to reduce lipid peroxidation in chicken muscle.

Soy isoflavones (ISF) have been shown to possess antioxidant activity (Wei et al., 1995; Ruiz-Larrea et al., 1997), which might be related to their anticancer, anti-inflammatory, and cardioprotective effects. It has been demonstrated that ISF decrease the concentrations of free radicals in plasma, liver, brain, testes, and kidney of male rabbits (Yousef et al., 2004). Cai and Wei (1996) suggested that dietary genistein, one of the two major component of ISF, enhances the activities of antioxidant enzymes in various organs of SENCAR mice. These observations led us to speculate that dietary ISF may improve growth performance or meat quality by increasing antioxidative status in animals.

The present study was conducted to investigate the effects of ISF on performance, meat quality and antioxidative activity in broiler chickens fed oxidized fish oil.

MATERIALS AND METHODS

Experimental protocol

One-day-old male broiler chickens (Lingnan yellow broiler, a quality meat-type chicken, coming into the market

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** Corresponding Author: Zong Yong Jiang. Tel: +86-20-8759 6262, Fax: +86-20-87503358, E-mail: jiangz38@hotmail.com

[2] Guangdong Public Laboratory of Animal Breeding and Nutrition, Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, P. R. China.

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Table 1. Ingredient and composition of the basal diets for the finishing broilers (as fed-basis)

Ingredients (%)	43 d to 63 d	Nutrients as calculation (%)	43 d to 63 d
Maize	69.80	ME (MJ/kg)[3]	12.96
Wheat bran	5.30	Crude protein	17.00
Fish meal (67.6%)	1.50	Lysine	0.85
Soy protein concentrate (63.5%)	9.10	Methionine+cystine	0.64
Maize gluten meal (60.1%)	5.00	Calcium	0.80
Fish oil[1]	3.00	Total phosphorus	0.56
Lysine·HCl	0.19	Non-phytate phosphorus	0.43
Methionine	0.03		
Limestone	1.22		
Dicalcium phosphate	1.08		
Salt	0.30		
Zeolite	2.48		
Vitamin-mineral premix[2]	1.00		
Total	100.00		

[1] Oxidized fish oil (acid value, 6.76 mg potassium hydroxide/g; peroxide value, 6.18 meq/kg).

[2] Supplied per kilogram of diet: riboflavin, 8.0 mg; niacin, 48 mg; pantothenic acid, 16 mg; 50% cholinechloride, 800 mg; cobalamin, 15 μg; cholecalciferol, 18.5 μg; vitamin E (DL-α-tocophery acetate), 20 IU; vitamin A (*trans*-retinyl acetate), 10,000 IU; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4 \cdot 7H_2O$, 300 mg; MnO, 100 mg; $CuSO_4 \cdot 5H_2O$, 20 mg; $ZnSO_4 \cdot H_2O$, 150 mg; $NaSeO_3$, 0.15 mg; KI, 0.5 mg; ethoxyquin, 100 mg; avoparcin, 15 mg. The carrier was zeolite.

[3] Values were calculated from data provided by Feed Database in China (2004).

at 63 d of age) were obtained from a commercial hatchery, and raised up to 42 d of age with free access to water and a commercial diet. At 42 d of age, a total 540 male broiler chickens were weighed and randomly allotted to 12 floor pens (1.3×3.5 m), 45 birds per pen. There were two dietary treatments (0 and 20 mg synthetic ISF/kg diet), with six replicates (pens) per treatment. The basal diet contained 3% oxidized fish oil, and did not contain soybean meal. Experimental diets were fed from 43 d to 63 d. Nutrient levels of the basal diets (Table 1) were based on NRC (1994). At 63 d of age, the birds were deprived of feed for 12 h and then weighed to get average daily gain (ADG), average daily feed intake (ADFI), and feed/gain data. To oxidize the fish oil, it was heated at 130°C for 5 h, which resulted in acid value 6.76 mg potassium hydroxide/g, and peroxide value 6.18 meq/kg.

At the end of feeding trial, 24 broilers, two birds per pen, were weighed and collected blood samples via wing vein. Blood samples were placed immediately on ice. Heparin was used as an anticoagulant. Plasma was obtained by centrifugation of samples at 860 g for 20 min. After that, the birds were sacrificed by cervical dislocation to get carcass traits data and breast meat samples. Plasma and some breast meat samples were stored at -70°C for biochemical analysis and enzyme analysis.

Measurements

The pH value was measured by direct probe of pH meter (HI8424, Beijing Hanna Instruments Science & Technology Co., Ltd, Beijing, P. R. China). The pH measurements were determined by thrusting probe pH meter into the right pectoralis major.

The water-holding capacity (WHC) of breast muscle was estimated 45 min postmortem by determining expressible juice using a modification of the filter paper press method described by Wierbicki and Deatherage (1958) as follows. A meat sample weighing about 1 g was placed between 18 pieces of 11-cm-diameter filter paper and pressed at 35 kg for 5 min. Expressed juice was defined as the loss in weight after pressing and presented as a percentage of the initial weight of the original sample (Bouton et al., 1971). Total moisture content was determined in duplicate according to AOAC procedures (AOAC, 1984). The WHC was calculated as the fraction of water retained by the meat (1-(expressible juice/total moisture content)) (Allen et al., 1998).

The colour measurements of breast muscles were carried out 45 min after slaughter to measure CIE lab values (L* measures relative lightness, a* relative redness, and b* relative yellowness) using a Chroma Meter (CR-410, Minolta Co., Ltd. Japan). A reading was made from the surface of sample, representing the whole surface of the muscle. A white tile (L*92.30, a*0.32, and b*0.33) was used as standard.

The whole left side of breast muscles was sampled and packed in plastic bags. These samples were refrigerated overnight at 4°C and then brought to room temperature before cooking. The breast muscle from each bird was cooked to an internal temperature of 70°C on a digital thermostat water bath (HH-4, Jiangbo instrument, Jiangsu, P. R. China). Endpoint internal temperature was monitored with a thermometer. Cooked muscle was cooled to room temperature. Slices of 1×1 cm were cut perpendicular to the fiber orientation of the muscle. Ten 1×1 cm cores about 3 cm thick were removed parallel to the fiber orientation through the thickest portion of the cooked muscle. Warner-

Table 2. Effects of soy isoflavone on growth performance and the carcass traits of male broilers fed oxidized fish oil

Item	Soy isoflavone (mg/kg)		p value
	0	20	
ADG (g/bird/d)	34.62±0.89[1]	35.15±0.64	0.64
ADFI (g/bird/d)	99.73±1.34	99.93±0.58	0.90
Feed/gain (g:g)	2.89±0.07	2.85±0.04	0.61
Mortality rate (%)	0.74±0.47	1.11±0.50	0.60
Breast muscle (%)[2]	11.90±0.34	11.92±0.25	0.97
Thigh muscle (%)[2]	16.17±0.19	16.07±0.21	0.73
Abdominal fat (%)[2]	2.07±0.17	2.04±0.21	0.91

[1] Means±SE (n = 6). [2] Percentage of live weight.

Bratzler shear force was determined by using an Instron Universal Mechanical Machine (Instron model 4411, Instron corp., Canton, MA, USA). A Warner-Bratzler apparatus was attached to a 50 kg load cell and tests were performed at a cross head speed of 127 mm/min. Signals were processed with the Instron Series 9th software package.

At slaughter, a slice of the muscle was isolated from the right side of breast to assess intramuscular fat content. The intramuscular fat content was determined using Soxhlet petroleum-ether extraction and expressed as the weight percentage of wet muscle tissue.

Biochemical analysis and enzyme analysis

Forty milligrams of frozen breast muscle in 4 ml homogenization buffer (0.05 M Tris-HCl, pH 7.4, 1 mM EDTA, 0.25 M sucrose) was homogenized on ice with an Ultra-Turrax (T8, IKA-Labortechnik, Staufen, Germany) for 5 sec at 13,500 rpm. The homogenate was centrifuged at 3,000 rpm for 10 min at 4°C, and the resultant supernatant was stored at -70°C until analysis. The activities of total superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, the total antioxidant capability (TAOC) were measured. In addition, the contents of lipid oxidation products, such as malondialdehyde (MDA) and lactic acid (LD), were analyzed using colorimetric methods with a spectrophotometer (Biomate 5, Thermo Electron Corporation, Rochester, NY, USA). The assays were conducted using the assay kits (Nanjing Jiancheng Insititute of Bioengineering, Nanjing, Jiangsu, P. R. China), and the procedures were followed accordingly. All samples were measured in triplicate, at appropriate dilutions, to give activities of the enzymes in the linear range of standard curves constructed with pure enzymes. Protein content of supernants was determined using the Coommasie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO, USA) assay with bovine serum albumin.

The lipid peroxidation was determined by measuring the amount of MDA via the thiobarbituric acid color reaction. The results were expressed as nmol MDA formed per milligram of protein (nmol/mgprot).

The LD production was measured by spectrophotometrically. The results were expressed as nmol LD formed per milligram of protein (nmol/mgprot).

The SOD activity was determined by monitoring the rate of inhibition of reduction of nitrite by the enzyme. The activity of SOD were expressed as unit per milligram of protein (U/mgprot). One unit represents the amount of enzyme required to produce 50% inhibition of nitrite reduction per min.

The catalase activity was measured spectrophotometrically by calculating the rate of degradation of H_2O_2 and expressed as unit per milligram of protein (U/mgprot). One unit represents 1 μmol H_2O_2 degradated per min.

The GPx activity was measured spectrophotometrically and expressed as unit per milligram of protein (U/mgprot). One unit represents the amount of enzyme required to decrease 1 μmol/L the content of glutathione per min.

The TAOC were measured by calculating the rate of reverting Fe^{3-} to Fe^{2-} and expressed as unit per milligram of protein (U/mgprot). One unit represents 0.01 gain of absorbance value per min.

MDA content, SOD, catalase, GPx activities and TAOC in plasma were analyzed by the above methods, the results were expressed as nmol/ml (MDA) or units per milliliter of plasma (U/ml).

Statistical analysis

All statistical analyses were computed using the GLM procedures of SAS software (SAS Institute Inc., 1996). A $p<0.05$ was considered statistically significant. Replicate was considered as the experimental unit for performance determined. The experimental unit was a bird for the other parameters. Numbers (n) used for statistics is noted in the tables. All data were expressed as means±SE.

RESULT AND DISCUSSION

Performance and carcass traits

In the present study, no differences were observed in ADG, ADFI, feed/gain, or carcass traits between the two treatments (Table 2). Wilhelms et al. (2006) reported that soy ISF supplemented into the diet at 1 and 5% did not influence the growth performance of the Japanese quail. Payne et al. (2001) reported that soy ISF levels in excess of those in a corn-soybean meal diet decreased gain: feed, and increased breast weight, but did not affect ADG or ADFI in broilers. It remains to be investigated, whether different doses and kinds of ISF compounds would affect the broiler growth performance and carcass traits or not.

Meat quality

The results of various meat characteristics were shown

Table 3. Effects of soy isoflavone on meat quality of male broilers fed oxidized fish oil

Item	Soy isoflavone (mg/kg)		p value
	0	20	
pH	5.78±0.07[1]	5.67±0.06	0.25
L*	60.96±1.176	60.65±1.186	0.86
a*	7.42±0.464[b]	8.80±0.431[a]	0.040
b*	11.24±0.872	9.53±0.588	0.11
WHC (%)[2]	58.65±3.566[b]	68.53±3.057[a]	0.047
Shear force (kgf)	3.081±0.183	2.811±0.120	0.22
Intramuscular fat (%)	0.232±0.019	0.231±0.018	0.98

[1] Means±SE (n = 12). [2] Water holding capacity. [a, b] $p<0.05$
L*, lightness; a*, redness; b*, yellowness of breast meat.

in Table 3. Compared to the control group, the a* value and WHC increased ($p<0.05$) by ISF supplementation. In breast muscle, broilers fed 20 mg ISF/kg had a 30.32% ($p<0.05$) reduction in LD production (Table 4).

The rate of discoloration of meat is believed to be related to the effectiveness of oxidation processes and enzymic reducing systems in controlling metmyoglobin levels in meat (Faustman and Cassens, 1989). This study indicated that ISF treatment increased meat color a* value significantly in male broilers fed oxidized fish oil. Similarly, Pace et al. (2006) reported that the prolonged administration of the subterranean clover ISF induced significant reductions of meat colour Lightness (L*) and Hue value in male sheeps. This implicated that the extent of myoglobin oxidation of muscle may be decreased by ISF supplementation.

In this study, the increased WHC by ISF treatment may be attributed to a decreased LD production (Lee et al., 1979; Raj et al., 1990; Raj et al., 1992). In postmortem muscle, the substrates glycogen, glucose, and glucose-6-phosphate are converted to lactate through anaerobic glycolysis. Lactate accumulation and the release of protons from adenosine triphosphate hydrolysis in postmortem muscle induce a pH decline (Bendall, 1973). A rapid pH decline may induce protein denaturation, resulting in decreased juiciness, and less intense (or pale) muscle coloration. Poultry meat with low pH has been associated with low WHC, which results in increased cook-loss and driploss (Allen et al., 1998; Qiao et al., 2001). The present results showed that the addition of synthetic ISF reduced the concentration of LD in breast muscles. Supplemental daidzein, one of the two major component of ISF, in the maternal diet during late gestation led to increases in longissimus muscle pH_{45} and pH_{end} in pigs from large litters (Rehfeldt et al., 2007). Therefore, the soy ISF supplementation would be expected to reduce meat oxidation and improve meat quality.

Lipid peroxidation and antioxidant enzymes

Table 4 show the content of MDA and the activities of SOD, CAT, GPx and TAOC in plasma and breast muscle of male broilers treated with ISF. As compared with the control, the activity of plasma catalase was increased ($p<0.05$) by supplementing with 20 mg ISF/kg diet. In breast muscle, broilers fed 20 mg ISF/kg had a 45.54% ($p<0.05$) decrease in MDA production. ISF supplementation significantly elevated the value of TAOC, activities of SOD and GPx by 210% ($p<0.05$), 35.73% ($p<0.05$), and 96.51% ($p<0.05$), respectively.

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and meat products. Oxidative damage to lipids occurs in the living animal because of an imbalance between the production of reactive oxygen species and the animal's defense mechanisms. This may be brought about by a high intake of oxidized lipids or poly-unsaturated fatty acids. Buckley et al. (1989) found that pork from pigs fed a diet containing 3% oxidized corn oil (peroxide value = 300 meq/kg of oil) was significantly more susceptible to oxidation than pork from pigs fed fresh

Table 4. Effects of soy isoflavone on biochemical parameters of plasma and breast muscle in male broilers fed oxidized fish oil

Item	Soy isoflavone (mg/kg)		p value
	0	20	
Plasma			
Malonedialdehyde (nmol/ml)	3.14±0.186[1]	3.15±0.215	0.97
Catalase (U/ml)	2.45±0.209[b]	3.14±0.175[a]	0.02
Glutathione peroxidase (U/ml)	2,874±139.7	2,924±208.1	0.85
Total superoxide dismutase (U/ml)	316.8±19.43	323.3±15.60	0.80
Total antioxidative capacity (U/ml)	10.42±0.78	10.34±0.776	0.94
Breast muscle			
Malonedialdehyde (nmol/mg protein)	0.51±0.097[a]	0.28±0.043[b]	0.04
Catalase (U/mg protein)	1.08±0.184	2.10±0.495	0.07
Glutathione peroxidase (U/mg protein)	0.95±0.090[b]	1.86±0.172[a]	0.002
Total superoxide dismutase (U/mg protein)	12.32±0.996[b]	16.72±1.182[a]	0.009
Total antioxidant capacity (U/mg protein)	0.020±0.002[b]	0.062±0.018[a]	0.029
Lactic acid (mmol/g protein)	1.260±0.082[a]	0.878±0.068[b]	0.002

[1] Means±SE (n = 12). [a, b] $p<0.05$.

corn oil. Jensen et al. (1997) reported that the oxidation of the dietary oil lowered lipid stability significantly in both raw and precooked meat. Buckley et al. (1989) suggested that the animal's natural defense system, namely GPx, may have been overwhelmed by the high dietary peroxides and, hence, allowed the passage of lipid peroxides through the intestinal mucosa. They also showed that oxidized dietary lipids had a destabilizing effect on muscle microsomes. The extent of lipid peroxidation can be monitored by MDA levels (Uuganbayar et al., 2005; Wang et al., 2006). This study indicated that MDA production of the breast muscle decreased by adding ISF in oxidized fish oil diet in male broilers. The ISF treatment was also observed suppressing lipid oxidation in male rabbits (Yousef et al., 2004) and hamsters (Fang et al., 2004). These findings suggested that ISF supplementation in broilers may protect tissues against lipid oxidation.

The ISF have multiple hydroxyl group(s) to act as effective antioxidants by donating a hydrogen atom(s) from their phenolic hydroxyl group(s) to peroxyradicals (Tikkanen et al., 1998). Diets containing ISF (150 and 250 ppm) showed obvious elevated antioxidant enzymatic levels in various organs of rats that were fed with diets containing partially oxidized oil (Liu et al., 2005). The antioxidant enzymes, catalase and GPx convert H_2O_2 to H_2O, and the SOD catalyzes the dismutation of the superoxide anion and prevents the subsequent formation of hydroxyl radicals (Imlay and Linn, 1988). In the present study, the increased activities of antioxidant enzymes indicated that ISF addition improved ability to detoxify H_2O_2 and the superoxide anion induced by the oxidized fish oil in plasma and meat. The antioxidant status of the subjects may be evaluated by measurement of TAOC (Tyssandier et al., 2004). The present results indicated that soy ISF improved antioxidative status in male broilers fed oxidized fish oil by elevating TAOC and activities of antioxidant enzymes.

In conclusion, the antioxidative status of chickens determined as TAOC, antioxidative enzymes, and the oxidative stability of lipids, increased by supplementing soy ISF to the diet containing oxidized fish oil. Increased antioxidative status obtained through soy ISF supplementation increased WHC and meat color redness (a*). In addition, the improved antioxidative status protected against lipid oxidation in breast muscles of soy ISF supplemented chickens. These results demonstrate that soy ISF shows good potential as an antioxidant in male broilers.

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