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Consumption of Oxidized Oil Increases Oxidative Stress in Broilers and Affects the Quality of Breast Meat

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A total of 120 4-week-old broiler chickens were allotted to 12 pens and fed one of three diets including control, oxidized diet (5% oxidized oil), or antioxidant-added diet (500 IU vitamin E) for 2 weeks. Blood samples were collected at the end of feeding trial, and breast muscles were sampled immediately after slaughter. Breast meats were also collected 24 h after slaughter and used for meat quality measurements. Oxidative stress in blood, lipid and protein oxidation, and sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity of breast muscle were determined. The oxidized diet increased oxidative stress in blood and increased carbonyl content in breast meat compared with the other two dietary treatments ($P < 0.05$). Lipid oxidation of breast muscles with the antioxidant-supplemented diet was lower than that with the oxidized and control diet groups ($P < 0.05$). Meat from birds fed the oxidized diet showed higher drip loss after 1 and 3 days of storage and greater 0−1 h post-mortem pH decline ($P < 0.05$). Significant differences in specific SERCA activity in breast muscles from birds fed control and oxidized diets ($P < 0.05$) were detected. This suggested that dietary oxidized oil induced oxidative stress in live birds and increased lipid and protein oxidation in breast muscle. Decrease in SERCA activity in breast muscles due to oxidative stress in live animals accelerated post-mortem glycolysis, which sped the pH drop after slaughter and increased drip loss, indicating that oxidation of diet can cause PSE-like (pale, soft, and exudative) conditions in broiler breast muscles.

KEYWORDS: Chicken; dietary oxidation; drip loss; protein oxidation; SERCA activity

INTRODUCTION

Fats and oils from animal and vegetable sources are used in feed formulation to increase the energy levels in a diet. Diets for broilers contain high levels of fat to meet the high energy requirements for fast growth. Some of the feed ingredients contain more fat or metal ions than others and different fatty acid compositions. Preparation of feeds involves mixing of various feed ingredients, which accelerates oxidative changes in feeds during preparation and storage. Therefore, the amount of oxidants in feeds can vary widely depending upon the ingredients used, fatty acid composition, amounts of antioxidants, and storage and temperature conditions of feeds before feeding (*1*).

Diet can be the main source of oxidants that all living animals have to face over their lifetime. "Oxidative stress" in an animal body occurs when the balance of formation or ingestion of oxidants exceeds the ability of antioxidant systems to remove reactive oxygen species (ROS) (*2*). Under these conditions, biomolecules are subjected to attack by excess ROS, and significant molecular and physiological damages can occur (*3*, *4*). Therefore, the degree of oxidative stress and molecular and physiological damages in animals can vary depending upon the degree of oxidation in their feed (*5*).

Lipid oxidation has been known to lead to quality problems in meat by forming off-odor and off-flavor compounds and decreasing nutritive values (*6*). In addition, the secondary products of lipid oxidation may react with proteins and lead to protein oxidation, which can cause fragmentation and conformational changes in the levels of secondary and tertiary structures to modify their functions (*7*). Oxidation-induced intermolecular bonds including disulfide, dityrosine, and other intermolecular bridges can lead to protein aggregation and polymerization to change protein proteolytic properties (*8*, *9*). These alterations can influence the physical and chemical properties of proteins including solubility, hydrophobicity, water-holding capacity, gelation functions, and even the nutritional value (*10*−*12*). Proteolytic activities of enzymes can be inhibited by protein oxidation, and enzyme oxidation has been reported to influence meat tenderness, color, and water-holding capacity in beef and pork (*13*−*15*). Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) is responsible for the uptake of calcium from cytoplasm to the sarcoplasmic reticulum of muscle cells. Therefore, SERCA activity is critical for the calcium concentration in the cytoplasm of muscle cells. High cytoplasm calcium levels may induce extreme muscle contraction, which further increases lactic

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acid production and pH drop in post-mortem muscle. Oxidation and nitration of SERCA can affect its activity and, thus, may lead to biochemical and physical changes in muscle during and after the slaughtering process (*16*).

However, only limited research has been conducted to study how oxidation in the diet is related to oxidative stress in living animals and to the biochemical reactions in muscle proteins and early post-mortem chicken muscles. Also, few studies if any have reported on whether these oxidation conditions in the diet can regulate meat quality including post-mortem pH changes and water-holding capacity in broiler breast meat. The objective of the current study was to investigate the effects of supplementing oxidized oil or antioxidants in diet on the oxidative stress of animals, SERCA activity, and the quality of fresh broiler breast meat.

MATERIAL AND METHODS

Animals and Feeds. One hundred and twenty 1-day-old broiler chicks were fed corn−soybean meal basal diet during the first 4 weeks. After 4 weeks, the birds were allotted to 12 pens and randomly distributed into 3 dietary treatments (**Table 1**). The three dietary treatments included control [basal diet containing 5% fresh animal/vegetable (AV) fat], oxidized diet (basal diet containing 5% oxidized AV fat), and antioxidants-added diet [basal diet containing 5% fresh AV fat, 500 IU vitamin E, and 200 ppm butylated hydroxyanisole (BHA)]. The control diet was prepared with a fresh AV fat blend with 25 IU vitamin E, the oxidized diet was prepared after oxidizing the same AV fat by exposure to room temperature until a peroxide value of 100 was attained, and the antioxidants-fortified diet was prepared with the fresh AV fat supplemented with BHA (200 ppm) and vitamin E (500 IU) per kilogram of feed. The AV fat was purchased from Feed Energy, Co., Des Moines, IA. The manufacturer has indicated that the sources of materials on the vegetable side are byproducts from soybean refineries and the animal fat sources are byproducts from packing plants. The AV fat was composed of 29% saturated, 37% monounsaturated, and 34% polyunsaturated fatty acids. The birds were housed in groups of 10 in 12 pens under standard conditions of temperature, humidity, and ventilation.

Sample Preparation and Storage. At the end of the experimental period, two birds from each pen were randomly selected to collect blood samples 1 day before slaughter following USDA guidelines (*17*). Immediately after slaughter, two birds from each pen were randomly selected, and one side of breast M. pecotoralis from each selected bird was sampled. The samples were frozen in liquid nitrogen and stored at −80 °C until analyzed for SERCA activity and lipid and protein oxidation. The other side of the breast muscle from the remaining carcasses was used to determine pH changes during the first 5 h after slaughter. The rest of the birds were chilled in ice water, drained, and stored in a 4 °C cold room for 24 h before deboning. Breast muscles were used to determine drip and cooking loss and color measurement. Blood samples were collected in a test tube containing anticoagulant (1 M EDTA) 1 day before slaughter from live broiler chickens. The blood samples were centrifuged at 1500*g* for 15 min, and the plasma was stored in a −20 °C freezer until analysis.

pH Measurement. pH was measured using a continuous method as described by Bendall (*18*). Breast muscle (3 g) was sampled in a test tube at 0, 1, 2.5, and 5 h postslaughter and homogenized with 27 mL of 150 mM KCl−5 mM iodoacetate solutions using a polytron (Brinkman Instruments Inc., Westbury, NY). The pH of the solution was measured with a pH-meter (Thermo Scientific, Beverly, MA). The rate of pH decline was calculated as the pH difference between two different post-mortem points to divide initial pH with the formula [(early pH − later pH)/early pH] × 100%.

Lipid Oxidation. Lipid oxidation was determined using a fluorometric thiobarbituric acid reactive substance (TBARS) method (*19*). For both plasma and homogenized meat, 0.5 mL of sample was placed in a test tube to which was added 200 μL of 8.1% SDS, 1.5 mL of 0.5 M HCl, 1.5 mL of 20 mM TBA in 15% trichloroacetic acid (TCA) solution, 50 μL of 7.2% butylated hydroxytoluene (BHT) in ethanol, and 250 μL of distilled water (DDW) and then vortex mixed thoroughly. The mixture was heated at 95 °C for 15 min in a water bath and cooled in ice for 10 min. To the sample was added 1 mL of DDW and 5 mL of a *n*-butanol/pyridine solution (1:1, v/v); the sample was vortex mixed thoroughly and centrifuged at 3000*g* for 15 min at room temperature. The upper layer was collected and used to measure the fluorescence by a fluorometer (model 450 digital fluorometer, Turner Corp., Dubuque, IA) with 520 nm excitation and 550 nm emission at gain 5.

Table 1. Ingredients and Composition of the Diets

	starter (1−2 weeks)	grower (3−4 weeks)	finisher (5−6 weeks)[a]
ingredient			
corn	56.826	63.954	64.131
soybean meal	35.98	29.111	27.27
AV fat	2.473	2.822	5
dicalcium phosphate	1.746	1.26	1.282
calcium carbonate	1.275	1.37	1.082
Iowa vitamin and mineral premix[b]	0.625	0.5	0.5
sodium chloride	0.462	0.462	0.463
methionine, 99%	0.266	0.258	0.184
biolysine, 50.7%	0.237	0.21	0.045
threonine, 99%	0.073		0.041
choline chloride, 60%	0.037	0.053	0.002
calculated analysis			
metabolizable energy, kcal/kg	3005	3100	3226.2
protein	22.48	19.71	18.7
total sulfur amino acids	0.98	0.9	0.8
methionine	0.61	0.57	0.48
lysine	1.34	1.14	1
arginine	1.45	1.24	1.18
glycine + serine	2.02	1.76	1.68
histidine	0.59	0.52	0.49
isoleucine	0.92	0.79	0.76
leucine	1.9	1.72	1.65
phenylalanine + tyrosine	1.35	1.17	1.12
threonine	0.91	0.73	0.74
valine	1.01	0.89	0.85
calcium	1	0.95	0.85
available phosphate	0.45	0.35	0.35
total phosphate	0.73	0.61	0.6
sodium	0.2	0.2	0.2

[a] Control diet: 5% fresh AV fat and Iowa vitamin and mineral premix. Oxidized diet: 5% oxidized AV fat (100 PV value). Antioxidant diet: 5% fresh AV fat and 500 IU + 200 ppm BHA per kg of diet. [b] Iowa vitamin and mineral premix supplies, per kilogram of diet, retinyl acetate, 8065 IU; cholecalciferol, 1580 IU; 25-hydroxycholecalciterol, 31.5 μg; DL-α-tocopheryl acetate, 25 IU; vitamin B_{12}, 16 μg; menadcre, 4 mg; riboflavin, 7.8 mg; pantothenic acid, 12.8 mg; niacin, 75 mg; choline chloride, 509 mg; folic acid, 1.62 mg; biotin, 0.27 mg; Mn, 80 mg; Zn, 90 mg; Fe, 60 mg; Cu, 12 mg; Se, 0.147 mg; sodium chloride, 2.247 g.

Protein Oxidation. Protein carbonyl content was determined using the 2,4-dinitrophenylhydrazine (DNPH) derivatization method (*20*). For muscle samples, 1 g of muscle was homogenized using a Polytron in 10 mL of pyrophosphate butter (2.0 mM $Na_4P_2O_7$, 10 mM Trizma-maleate, 100 mM KCl, 2.0 mM $MgCl_2$, and 2.0 mM EGTA, pH 7.4). After homogenization, two equal aliquots of solutions (2 mL) were precipitated with 2 mL of 20% TCA and centrifuged at 12000*g* for 5 min. For blood plasma, two equal aliquots of 0.5 mL of sample were directly precipitated with 20% TCA and centrifuged. The supernatant of sample was discarded, and the precipitant was further washed with 2 mL of 10% TCA followed by centrifugation at 12000*g* for 5 min. After centrifugation, one pellet was treated with 2 mL of 10 mM DNPH dissolved in 2 M HCl, and the other pellet was incubated with 2 M HCl as a blank. During 30 min of incubation, the sample was vortex mixed for 10 s every 10 min. The proteins were further precipitated with 2 mL of 20% TCA and centrifuged at 12000*g* for 5 min. DNPH was removed by washing the sample three times with 5 mL of 10 mM HCl in 1:1 (v/v) ethanol/ethyl acetate followed by centrifugation at 12000*g* for 5 min after each wash. The pellet was finally solubilized in 2 mL of 6.0 M guanidine hydrochloride dissolved in 20 mM potassium dihydrogen phosphate (pH 2.3 adjusted with trifluoroacetic acid). The samples were kept at 4 °C overnight, and the final solution was centrifuged to remove insoluble materials the following day. The absorbance was measured using a spectrophotometer at 370 nm. The absorbance values of blank samples were subtracted from their corresponding sample values. The carbonyl content was calculated as nanomoles per milligram of protein using an absorption coefficient of 22000 M^{-1} cm^{-1} (*21*).

Drip Loss. Drip loss was measured using whole breast muscles. Whole breast samples were placed in individual plastic bags under atmospheric conditions at 4 °C. Immediately prior to being placed in bags, breast samples were dried by towel, and the initial weights were recorded. One day after storage, samples were removed from their individual bags, towel dried, and weighed again. The breasts were then placed in new bags and stored for an additional 2 days. Following 3 days of storage, breast samples were again towel dried and weighed. Drip loss after 1 or 3 days of storage was calculated as the difference between the final and initial weights expressed as a percentage of the initial weight: [(initial weight − final weight)/initial weight] × 100%.

Water-Holding Capacity. A centrifugal method was used to measure the water-holding capacity as reviewed by Trout (*22*). Fifteen grams of minced breast samples was placed in a 35 mL test tube and centrifuged for 10 min at 40000*g*. The liquid in the tube was removed, and the meat sample was weighed. The percentage of water lost was recorded as a drip loss.

Color Measurement. Color measurements for breast muscles were conducted 1 day after slaughter using a HunterLab MiniScan XE colorimeter (Hunter Laboratory Inc., Reston, VA) with D65 illuminant and 10° standard observer. The instrument was calibrated against blank and white references prior to use. Four random readings per sample were taken and averaged for CIE L^* (lightness), a^* (redness), and b^* (yellowness) values.

Sarcoplasmic Reticulum Ca^{2+}-ATPase (SERCA) Activity. Sarcoplasmic reticulum (SR) was isolated from breast muscles, and the SERCA activity was determined using the method described by Dremina et al. (*23*) with minor modifications. Breast muscle (10 g) were homogenized with 30 mL of pH 7.4 buffer containing 0.1 M KCl, 0.1 mM EDTA, and 20 mM Mops using a polytron at maximal speed. After homogenization, samples were centrifuged at 5000*g* for 20 min to remove cell debris. The supernatant was collected, and the pellet was re-extracted and centrifuged. To precipitate mitochondria, pooled supernatant was recentrifuged at 11800*g* for 10 min. Collected supernatant was filtered through a Whatman no. 1 filter paper (GE Healthcare, Piscataway, NJ) followed by the addition of KCl to a final concentration of 0.6 M. The samples were incubated at 4 °C for 20 min and centrifuged at 23500*g* for 1 h to pellet SR. The SR pellets were suspended in a pH 7.0 buffer containing 0.3 M sucrose and 20 mM Mops for 1 h and centrifuged again at 40000*g* for 1 h. SR vesicles were collected and homogenized with 0.3 M sucrose and 20 mM Mops (pH 7.0), and aliquots were quickly frozen using liquid nitrogen. The protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA), and the samples were stored at −80 °C.

Ca^{2+}-dependent and basal ATPase activities of SERCA in SR were measured by NADH degradation in the presence or absence of calcium ionophore thapsigargin (*24*, *25*). Briefly, 1 mM ATP (final concentration) was added to a 1.5 mL (pH 7.0) solution containing 10 μg of SR, 4.9 mM NaN_3, 0.06 mM EGTA, 100 mM KCl, 3 mM $MgCl_2$, 0.2 mM NADH, 1 mM phosphoenol pyruvate, 12.6 units of pyruvate kinase, 18 units of lactate dehydrogenase, 21 mM Mops, and 0.01 mM $CaCl_2$. Nonspecific SERCA activity was analyzed in the mixture with 0.01 mM calcium in the presence of 0.1 μM thapsigargin. The rate of NADH degradation was measured with a spectrophotometer at 340 nm.

Statistical Analysis. All data were analyzed using SAS version 9.1 (Cary, NC), and significance was reported at $P < 0.05$ level. The general linear procedure and analysis of variance (ANOVA) were used to determine the significance of the effects of diets.

RESULTS AND DISCUSSION

Effects of Dietary Treatments on Performance of Broiler Chickens. Dietary treatments did not show significant effects on weight gain of broiler chickens between 4 and 6 weeks ($P > 0.05$, **Table 2**). Feed consumption of birds between 4 and 6 weeks was not significantly different among three treatments ($P > 0.05$). No significant difference was found for feed efficiency (weight gain/feed intake) during the experiment period ($P > 0.05$).

Dietary oxidation may impair the energy and the nutritional value of oil or fat, which possibly decreases the efficiency of feed utilization. Previous studies have shown that feeding rancid rice bran significantly lowered weight gain and feed efficiency in broiler chickens during 4−6 weeks (*26*). Lin et al. (*27*) fed broiler chickens a diet containing 5.5% oxidized sunflower oil (peroxide value of 400 mequiv/kg of oil) for 4 weeks and found that the carcass weights of the broilers fed oxidized oil were 7.3% lower than those from a control group. However, no significant difference was found in feed efficiency, feed consumption, and dressing percent. No significant differences in growth performance and feed consumption in the current study may be partly due to the shorter feeding period with oxidized oil and lower oxidation levels of oil than in other studies (*26*, *27*). The effects of vitamin E on the growth performance of broiler chickens highly depended upon the level and duration of vitamin E supplementation and dietary composition (*28*). No significant differences in growth performance and feed consumption of broiler chickens were detected between control and antioxidant-supplemented group in this study (**Table 2**)

Table 2. Effects of Dietary Treatments on Growth and Feed Consumption in Broiler Chickens

	control	oxidized oil	antioxidant
4 week weight, kg	1.371 ± 0.030	1.415 ± 0.028	1.339 ± 0.010
6 week weight, kg	2.743 ± 0.068	2.778 ± 0.065	2.669 ± 0.039
weight gain, kg	1.372 ± 0.048	1.362 ± 0.039	1.331 ± 0.029
4−6 week feed intake, kg	2.387 ± 0.049	2.419 ± 0.075	2.320 ± 0.038
gain/feed, kg/kg	0.574 ± 0.011	0.563 ± 0.003	0.573 ± 0.003

Table 3. Effects of Dietary Treatments on Carbonyl Content (Nanomoles per Milligram of Protein) and Lipid Oxidation (Fluorometric Reading) in Blood and Breast Muscle Samples[a]

	control	oxidized oil	antioxidant
blood samples			
protein oxidation	0.31 ± 0.03	0.38 ± 0.02	0.33 ± 0.03
lipid oxidation	15.38 ± 0.42 b	18.75 ± 0.45 c	14.14 ± 0.34 a
breast muscle samples			
protein oxidation	0.55 ± 0.05 a	0.70 ± 0.05 b	0.55 ± 0.04 a
lipid oxidation	19.60 ± 3.08 a	27.40 ± 3.40 b	13.00 ± 0.62 a

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

Effects of Dietary Treatments on Protein and Lipid Oxidation in Blood and Breast Muscle. Dietary oxidation may destroy fat-soluble vitamins and increase their susceptibility to lipid oxidation in the gastrointestinal tract or other tissues (*29*). Dietary supplementation with 5% oxidized oil resulted in higher levels of lipid oxidation in blood plasma than in the control group ($P < 0.05$) (**Table 3**). This result was in agreement with the study of Engberg et al. (*30*), who reported that inclusion of 11% oxidized vegetable oil in broiler diets caused higher levels of TBARS and lower concentration of α-tocopherol, β-carotene, and lutein in blood plasma compared to chickens fed fresh oil. Addition of vitamin E and BHA in the diet showed significant effects on lowering the level of lipid oxidation compared to oxidized oil treatment ($P < 0.05$) in blood (**Table 3**). These results suggested that feeding broilers oxidized oil increased the oxidative stress in vivo. The TBARS value of breast muscle from animals fed a diet with added oxidized oil was significantly higher than those from control and vitamin E groups ($P < 0.05$) (**Table 3**). Jensen et al. (*31*) fed broilers a highly oxidized rapeseed and soybean oil for 4 weeks and found that oxidation of dietary oil resulted in decreased lipid stability in both raw and precooked meat during chilled storage. The TBARS values of meat from the oxidized oil group increased significantly more quickly than the control group in both thigh and breast meats (*31*). The increased levels of lipid oxidation in breast samples from the oxidized group may be due

Table 4. Effects of Dietary Treatments on Drip and Cooking Loss in Broiler Breast Meat[a]

	control	oxidized oil	antioxidant
day 1 drip loss, %	0.422 ± 0.053 a	0.687 ± 0.067 b	0.538 ± 0.057 a
day 3 drip loss, %	0.794 ± 0.085 a	1.372 ± 0.164 b	0.934 ± 0.091 a
day 1 water-holding capacity, %	2.79 ± 0.25 a	4.74 ± 0.69 b	4.25 ± 0.63 ab
cooking loss, %	21.20 ± 0.81	21.50 ± 0.97	20.23 ± 1.18

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

to the decreased accumulation of α-tocopherol, which could have been denatured by feeding oxidized oil (*32*).

The secondary products derived from lipid oxidation can interact with the amino acids of proteins, and these interactions can regulate protein structure and function. For example, lipid oxidation products including hydroperoxides and aldehydes can transform lysine residues into pyrrole derivatives (*33*). The derivatives of interactions between lipid oxidation products and amino acid residues can cause formation of cross-linkage between proteins (*34*). However, no research has been conducted to determine the effects of dietary oxidized oil on protein oxidation in broiler chicken meat.

The blood of birds fed oxidized oil tended to have higher levels of carbonyl content than control ($P = 0.08$) and antioxidant ($P = 0.10$) groups (**Table 3**). Higher carbonyl content was detected in breast muscles from the oxidized group than the control and vitamin E groups ($P < 0.05$). However, no significant difference was found for protein oxidation between control and antioxidant groups in both blood and breast samples (**Table 3**). Previous studies on the effects of dietary vitamin E on protein oxidation were inconsistent. Mercier et al. (*35*) reported that feeding turkeys a diet supplemented with 400 mg of tocopheryl acetate/kg of feed for 16 weeks showed no significant difference in the amount of carbonyl content in M. pectoralis during refrigerated storage. However, the same study reported higher content of thiol group in muscles from vitamin E supplemented group than control group (*35*). In microsomal membranes from turkey muscles, metmyoglobin and hydrogen peroxide were utilized to generate oxidative conditions (*36*). Vitamin E supplementation was shown to be effective in lowering TBARS and lipofuscins, whereas no significant effects were detected for preventing protein oxidation and scavenging radical formation in muscle (*36*).

Effects of Dietary Treatments on Meat Color, Drip Loss, and Cooking Loss. The breast meat of broiler chickens fed a diet containing oxidized oil showed significantly higher drip loss than the control group after 1 day of storage under atmospheric conditions at 4 °C ($P < 0.01$, **Table 4**). The drip loss of meat at day 1 from the oxidized oil group was 63 and 44% higher than that of the control and antioxidant-supplemented groups, respectively. This tendency was also detected after 3 days of storage. The control and antioxidant-supplemented group had significantly lower drip loss than the oxidized oil group ($P < 0.01$). The water-holding capacity, measured by water loss during high-speed centrifugation, of breast muscle from oxidized oil group was lower compared to the control ($P < 0.05$) at day 1. However, no significant differences in drip loss were found between control and antioxidant-supplemented diet groups after 1 and 3 days of storage ($P > 0.05$). Cooking loss was also not significantly different among the three dietary treatments ($P > 0.05$) (**Table 4**).

Pale, soft, and exudative (PSE) condition has been extensively studied in pork. However, PSE-like studies have emerged in poultry only during the past decade (*37*). The fundamental biochemical events for PSE in poultry have not yet been well understood, although mechanisms similar to those in pork PSE should be involved (*38*). The effect of dietary vitamin E on drip loss in broiler chicken breast meat is very limited. With other species, the results were inconsistent due to differences in the amounts of vitamin E supplemented, muscle compositions, and stability of mitochondria and sarcoplasmic reticulum among studies (*39*).

Table 5. Effects of Dietary Treatment on Broiler Breast Meat Color[a]

	control	oxidized oil	antioxidant
L^* value	63.6 ± 2.3	63.0 ± 3.1	63.7 ± 3.1
a^* value	9.3 ± 1.1	9.6 ± 1.9	9.5 ± 1.3
b^* value	12.6 ± 2.3	12.2 ± 1.9	12.2 ± 2.1

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

Table 6. Effects of Dietary Treatments on pH Changes in Broiler Breast Muscle[a]

	control	oxidized oil	antioxidant
0 h	6.75 ± 0.03	6.80 ± 0.02	6.78 ± 0.02
1 h	6.68 ± 0.02	6.62 ± 0.02	6.67 ± 0.04
2.5 h	6.43 ± 0.03	6.41 ± 0.04	6.38 ± 0.03
5.0 h	6.12 ± 0.02	6.11 ± 0.02	6.12 ± 0.05
0−1 h decline, %	1.38 ± 0.34 a	2.65 ± 0.14 b	1.62 ± 0.30 a
0−2.5 h decline, %	6.18 ± 0.35	6.72 ± 0.49	6.41 ± 0.71
0−5 h decline, %	9.99 ± 0.91	10.53 ± 0.20	9.53 ± 1.10

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

The color L^* (lightness), a^* (yellowness), and b^* (brownness) values of breast muscle from three diets did not differ significantly in the current study ($P > 0.05$, **Table 5**). This result was consistent with the pH of breast muscle, which showed no significant differences among the three diet groups at 0, 1, 2.5, and 5 h post-mortem. Mercier et al. (*40*) reported that the color of breast muscle from broiler chickens was influenced by meat pH. Low pH could scatter more light back, resulting in pale color, whereas high pH could allow light to be transmitted into the deep section of meat, leading to dark color. Although vitamin E supplementation in diet influenced the color of post-mortem pork and beef, poultry breast meat color was not affected by dietary vitamin E (*35*, *41*).

Effects of Dietary Treatments on the Extent and Rate of pH Decline in Post-mortem Muscle. The post-mortem pH values at 0, 2.5, and 5 h were not significantly different among three dietary treatments (**Table 6**). However, the pH of breast muscle from birds fed a diet containing oxidized oil tended to be lower than those from the control and antioxidant groups ($P = 0.10$) at 1 h post-mortem. The rate of pH decline between 0 and 1 h post-slaughter in breast muscle from birds fed oxidized diet was faster than that from the other two groups ($P < 0.05$), but the rates of pH decline at 0−2.5 and 0−5 h were not significant ($P > 0.05$). The faster rate of pH decline early post-mortem (0−1 h) in the breast muscles from oxidized diet may partly explain the higher drip loss and lower water-holding capacity in that group. This is due to the fact that a fast rate of pH decline or low pH plus high body temperature in the early post-mortem stage can lead to the denaturation of muscle proteins (*42*). The denaturation of myofibrillar proteins can result in the loss of their functionality, which further decreases their water-holding capacity. For example, pH-induced myosin denaturation can result in the shrinkage of myosin head, and this shrinkage can decrease the space between myofibrillar filaments to force the water out of myofibrils and increase drip loss (*43*). The higher drip loss or lower water-holding

Table 7. Effects of Dietary Treatments on Nonspecific and Specific SERCA Activity (Micromoles of Pi per Milligram of Protein per Minute) in Broiler Breast Muscle[a]

	control	oxidized oil	antioxidant
nonspecific activity	298.03 ± 60.62	222.20 ± 25.68	313.75 ± 56.49
activity at 0.01 mM calcium	315.59 ± 38.90 b	229.59 ± 26.87 a	342.58 ± 19.94 b
activity at 0.02 mM calcium	553.22 ± 54.97 b	378.63 ± 33.62 a	479.42 ± 49.80 ab

[a] Means within the same row with different letters are significantly different ($P < 0.05$).

capacity in broiler chickens from the oxidized group also could be due to the higher levels of protein oxidation in that group. Protein oxidation could change the structure and biochemical function of proteins by fragmentation, aggregation, and polymerization (*8*,*9*). For example, the oxidation of aromatic residues can lead to the addition of some charged groups, which increases the hydrophilic status for these residues. These changes may force oxidized aromatic residues to be present on the protein surface, resulting in conformational changes of protein (*44*). After myofibrillar proteins from turkey white muscle were incubated with 25 μM iron and copper, the water-holding capacity of gels made from these proteins was lowered by 10 and 23%, respectively (*45*).

Effects of Dietary Treatments on SERCA Activity. Addition of oxidized oil in the diet lowered the specific SERCA activity measured in the calcium level 0.01 and 0.02 mM at pH 7 ($P < 0.05$). However, no significant difference in nonspecific and specific SERCA activity between the control and antioxidant-supplemented groups ($P < 0.05$) was detected (**Table 7**). The lower SERCA activity might be caused by the increased oxidative stress, which resulted in SERCA oxidation, in the birds fed a diet containing oxidized oil. In addition, lower deposition of antioxidant in the breast muscle of the oxidized group could have decreased its ability to maintain the antioxidant system, leading to increased accumulation of reactive nitrogen and oxygen species.

SERCA is responsible for calcium uptake from cytoplasm into sarcoplasmic reticulum, and inactivation of SERCA is related to increased levels of calcium in the cytoplasm of skeletal muscle cells (*46*). Increased release of calcium from sarcoplasmic reticulum can cause extreme muscle contraction and increase the production of lactate, which further increases the rate and extent of pH decline during the conversion of muscle to meat postmortem. SERCA contains 24−29 cysteines per molecule depending on isoforms, and thus SERCA is highly sensitive to oxidation and nitrosylation (*47*). Klebl et al. (*16*) reported that SERCA activity was inactivated by protein oxidation and tyrosine nitration in rabbit muscle (*48*). Instead of inhibiting SERCA activity, oxidation and nitrosylation may also regulate the activity of the ryanodine receptor calcium release channel (RyR), which is responsible for calcium release from the sarcoplasmic reticulum to cytosol of muscle cells. Nitrosylation of RyR could cause leaky channels for calcium, leading to a rapid rise of calcium concentration in the sarcoplasm of muscle cells (*49*). Therefore, increased activity of RyR and decreased SERCA activity can eventually cause a faster rate of pH decline, lower ultimate pH, and increased drip loss during chilling and storage.

In conclusion, oxidation of lipids in a diet did not show significant effects on the growth performance and feed efficiency of broiler chickens. However, dietary oxidation can lead to deteriorated changes in meat quality, especially water-holding capacity, during post-mortem refrigerated storage. The decreased meat quality may be related to the biochemical changes including protein and lipid oxidation in the early post-mortem stage. These changes may be due to the increased oxidative stress in live animals and the decreased accumulation of antioxidant components in tissues, which may have resulted in high levels of reactive oxygen and reactive nitrogen species in post-mortem muscle.

LITERATURE CITED

(1) Warnants, N.; VanOeckel, M. J.; Boucque, C. V. Incorporation of dietary polyunsaturated fatty acids in pork tissues and its implications for the quality of the end products. *Meat Sci.* **1996**, *44*, 125−144.

(2) Urso, M. L.; Clarkson, P. M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* **2003**, *189*, 41−54.

(3) Wetzstein, C. J.; Shern-Brewer, R. A.; Santanam, N.; Green, N. R.; White-Welkley, J. E.; Parthasarathy, S. Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? *Free Radical Biol. Med.* **1998**, *24* (4), 679−682.

(4) Betti, M.; Schneider, B. L.; Wismer, W. V.; Carney, V. L.; Zuidhof, M. J.; Renema, R. A. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poult. Sci.* **2009**, *88*, 1085−1095.

(5) Lauridsen, C.; Nielsen, J. H.; Henckel, P.; Sorensen, M. T. Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E, and copper. *J. Anim. Sci.* **1999**, *77* (1), 105−115.

(6) Kanner, J. Oxidative processes in meat and meat products: quality implications. *Meat Sci.* **1994**, *36*, 169−189.

(7) Hidalgo, F. J.; Alaiz, M.; Zamora, R. Effect of pH and temperature on comparative nonenzymatic browning of protein's produced by oxidized lipids and carbohydrates. *J. Agric. Food Chem.* **1999**, *47*, 742−747.

(8) Martinaud, A.; Mercier, R.; Marinova, P.; Tassy, C.; Gatellier, P.; Renerre, M. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. *J. Agric. Food Chem.* **1997**, *45*, 2481−2487.

(9) Morzel, M.; Gatellier, P. H.; Sayd, T.; Renerre, M.; Laville, E. Chemical oxidation decreases proteolytic susceptibility of skeletal muscle proteins. *Meat Sci.* **2006**, *73*, 536−543.

(10) Liu, G.; Xiong, Y. L. Electrophoretic pattern, thermal denaturation, and in vitro digestibility of oxidized myosin. *J. Agric. Food Chem.* **2000**, *48*, 624−630.

(11) Srinivasan, S.; Hultin, H. O. Chemical, physical and functional properties of cod proteins modified by a non enzymatic free-radical generating system. *J. Agric. Food Chem.* **1997**, *45*, 310−320.

(12) Srinivasan, S.; Xiong, Y. L. Gelation of beer heart surimi as affected by antioxidants. *J. Food Sci.* **1996**, *61*, 707−711.

(13) Rowe, L. J.; Maddock, K. R.; Lonergan, S. M.; Huff-Lonergan, E. Influence of early postmortem protein oxidation on beef quality. *J. Anim. Sci.* **2004**, *82*, 785−793.

(14) Rowe, L. J.; Maddock, K. R.; Lonergan, S. M.; Huff-Lonergan, E. Oxidation environments decrease tenderization of beef steaks through inactivation of μ-calpain. *J. Anim. Sci.* **2004**, *82*, 3254−3266.

(15) Lund, M. N.; Lametsch, R.; Hviid, M. S.; Jensen, O. N.; Skibsted, L. J. High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine *longissimus dorsi* during chill storage. *Meat Sci.* **2007**, *77*, 295−303.

(16) Klebl, B. M.; Ayoub, A. T.; Pette, D. Protein oxidation, tyrosine nitration, and inactivation of sarcoplasmic reticulum Ca^{2+}-ATPase in low-frequency stimulated rabbit muscle. *FEBS Lett.* **1998**, *422*, 381−384.

(17) USDA. Guidelines for establishing and operating broiler processing plants. *Agricultural Handbook No. 581*; USDA-ARS: Washington, DC, 1982.

(18) Bendall, J. R. Postmortem changes in muscle. In *The Structure and Function of Muscle*; Bourne, G. H., Ed.; Academic Press: New York, 1973; Vol. II, Part 2, pp 244−309.

(19) Jo, C.; Ahn, D. U. Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poult. Sci.* **1998**, *77*, 475−480.

(20) Lund, M. N.; Hviid, M. S.; Claudi-Magnussen, C.; Skibsted, L. H. Effects of dietary soybean oil on lipid and protein oxidation in pork patties during chill storage. *Meat Sci.* **2008**, *79*, 727−733.
(21) Levine, R. L.; Williams, J. A.; Stadtman, E. R.; Shacter, E. Carbonyl assays for determination of oxidatively modified proteins. *Methods Enzymol.* **1994**, *233*, 346−357.
(22) Trout, G. R. Techniques for measuring water-binding capacity in muscle foods − a review of methodology. *Meat Sci.* **1988**, *23*, 235−252.
(23) Dremina, E. S.; Sharov, V. S.; Kumar, K.; Zaid, A.; Michaelis, E. K.; Schöneich, C. Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). *Biochem. J.* **2004**, *383*, 361−370.
(24) Chu, A.; Dixon, M. C.; Saito, A.; Seiler, S.; Fleischer, S. Isolation of sarcoplasmic reticulum fraction referable to longitudinal tubules and junctional terminal cisternae from rabbit skeletal muscle. *Methods Enzymol.* **1988**, *157*, 36−46.
(25) Bupha-Intr, T.; Wattanapermpool, J. Regulatory role of ovarian sex hormones in Calcium uptake activity of cardiac sarcoplasmic reticulum. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *291*, H1101−H1108.
(26) Chae, B. J.; Lee, K. H.; Lee, S. K. Effects of feeding rancid rice bran on growth performance and chicken meat quality in broiler chicks. *Asian Aust. J. Anim. Sci.* **2002**, *15*, 266−273.
(27) Lin, C. F.; Asghar, A.; Gray, J. I.; Buckley, D. J.; Booren, A. M.; Crackel, R. L.; Flegal, C. J. Effects of oxidised dietary oil and antioxidant supplementation on broiler growth and meat stability. *Br. Poult. Sci.* **1989**, *30*, 855−864.
(28) Sheehy, P. J. A.; Morrissey, P. A.; Flynn, A. Influence of dietary α-tocopherol on tocopherol concentrations in chick tissues. *Br. J. Nutr.* **1991**, *32*, 391−397.
(29) Sheehy, P. J. A.; Morrissey, P. A.; Flynn, A. Consumption of thermally-oxidized sunflower oil by chicks reduces α-tocopherol status and increases susceptibility of tissues to lipid oxidation. *Br. J. Nutr.* **1994**, *71*, 53−65.
(30) Engberg, R. M.; Lauridsen, C.; Jensen, S. K.; Jakobsen, K. Inclusion of oxidized vegetable oil in broiler diets. Its influence on nutrient balance and on the antioxidative status of broilers. *Poult. Sci.* **1996**, *75*, 1003−1011.
(31) Jensen, C.; Engberg, R.; Jakobsen, K.; Skibsted, L. H.; Bertelsen, G. Influence of the oxidative quality of dietary oil on broiler meat storage stability. *Meat Sci.* **1997**, *47*, 211−222.
(32) Liu, J. F.; Huang, C. J. Dietary oxidized frying oil enhances tissue α-tocopherol depletion and radioisotope tracer excretion in vitamin E-deficient rats. *J. Nutr.* **1996**, *126*, 2227−2235.
(33) Hidalgo, F. J.; Alaiz, M.; Zamora, R. A spectrophotometric method for the determination of protein damaged by oxidized lipids. *Anal. Biochem.* **1998**, *262*, 129−136.
(34) Zamora, R.; Alaiz, M.; Hidalgo, F. J. Contribution of pyrrole formation and polymerization to the nonenzymatic browning produced by amino−carbonyl reactions. *J. Agric. Food Chem.* **2000**, *48*, 3152−3158.
(35) Mercier, Y.; Gatellier, P.; Viau, M.; Remignon, H.; Renerre, M. Effect of dietary fat and vitamin E on colour stability and on lipid and protein oxidation in turkey meat during storage. *Meat Sci.* **1998**, *48*, 301−318.
(36) Batifoulier, F.; Mercier, Y.; Gatellier, P.; Renerre, M. Influence of vitamin E on lipid and protein oxidation induced by H_2O_2-activated MetMb in microsomal membranes from turkey muscle. *Meat Sci.* **2002**, *61*, 389−395.
(37) Barbut, S.; Sosnicki, A. A.; Lonergan, S. M.; Knapp, T.; Ciobanu, D. C.; Gatcliffe, L. J.; Huff-Lonergan, E.; Wilson, E. W. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat Sci.* **2008**, *79*, 46−63.
(38) Solommon, M. B.; Van Laack, R. L. J. M.; Eastridge, J. S. Biophysical basis of pale, soft, exudative (PSE) pork and poultry muscle: a review. *J. Muscle Foods* **1998**, *9*, 1−11.
(39) den Hertog-Meischke, M. J. A.; Smulders, F. J. M.; Houben, J. H.; Eikelenboom, G. The effect of dietary vitamin E supplementation on drip loss of bovine *Longissimus Lumborum*, *Psoas Major* and *Semitendinosus* muscles. *Meat Sci.* **1997**, *45*, 153−160.
(40) Swatland, H. J. How pH causes paleness or darkness in chicken breast meat. *Meat Sci.* **2008**, *80*, 396−400.
(41) Zouari, N.; Elgharbi, F.; Fakhfakh, N.; Ben Bacha, A.; Garouri, Y.; Miled, N. Effect of dietary vitamin E supplementation on lipid and colorstability of chicken thigh meat. *Afr. J. Biotechnol.* **2010**, *9*, 2276−2283.
(42) Huff-Lonergan, E.; Lonergan, S. M. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* **2005**, *71*, 194−204.
(43) Offer, G.; Cousins, T. The mechanism of drip production-formation of 2 compartments of extracellular-space in muscle postmortem. *J. Sci. Food Agric.* **1992**, *58*, 107−116.
(44) Chao, C.; Ma, Y.; Stadtman, E. R. Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2969−2974.
(45) Decker, E. A.; Xiong, Y. L.; Calvert, J. T.; Crum, A. D.; Blanchard, S. P. Chemical, physical, and functional properties of oxidized turkey white muscle myofibrillar proteins. *J. Agric. Food Chem.* **1993**, *41*, 186−189.
(46) Jorgensen, P. L.; Andersen, J. P. Structural basis for E1-E2 conformational transition in Na, K-pump and Ca-pump proteins. *J. Membr. Biol.* **1988**, *103*, 97−120.
(47) Zhao, M.; Lytton, J.; Burchiel, S. W. Inhibition of sarco-endoplasmic reticulum calcium ATPase (SERCA) by polycyclic aromatic hydrocarbons: lack of evidence for direct effects on cloned rat enzymes. *Int. Soc. Immunopharm.* **1996**, *18*, 589−598.
(48) Renerre, M.; Dumont, F.; Gatellier, P. Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. *Meat Sci.* **1996**, *43*, 111−121.
(49) Bellinger, A. M.; Reiken, S.; Dura, M.; Murphy, P. W.; Deng, S. X.; Landry, D. W.; Nieman, D.; Lehnart, S. E.; Samaru, M.; LaCampagne, A.; Marks, A. R. Remodeling of ryanodine receptor complex causes "leaky" channels: a molecular mechanism for decreased exercise capacity. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 2198−2202.

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