Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity[1]

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ABSTRACT Recent nutrigenomic studies have shown that animal nutrition can have a major influence on tissue gene expression. Dietary antioxidant supplements can enhance the quality of meat through modification of tissue metabolic processes. This study investigated the influence of dietary antioxidants and quality of oil on the oxidative and enzymatic properties of chicken broiler breast meat stored in an oxygen-enriched package (HiOx: 80% O_2/20% CO_2) in comparison with air-permeable polyvinylchloride (PVC) or skin packaging systems during retail display at 2 to 4°C for up to 21 d. Broilers were fed either a diet with a low-oxidized (peroxide value 23 mEq of O_2/kg) or high-oxidized (peroxide value 121 mEq of O_2/kg) oil, supplemented with or without an algae-based Se yeast and organic mineral antioxidant pack for 42 d. Lipid and protein oxidation and tissue enzymatic activity were analyzed. In all packaging systems, lipid oxidation (TBA reactive substances) was inhibited by up to 32.5% ($P < 0.05$) with an antioxidant-supplemented diet when compared with diets without antioxidants, particularly in the HiOx and PVC systems. Protein sulfhydryls were significantly protected by antioxidant diets (e.g., by 14.6 and 17.8% for low-and high-oxidized dietary groups, respectively, in PVC d 7 samples). Glutathione peroxidase, catalase, and superoxide dismutase activities were significantly higher ($P < 0.05$) in antioxidant-supplemented diets compared with the basal diet, regardless of oil quality. Also, serum carbonyls were lower in broilers fed a low-oxidized antioxidant-supplemented treatment. The results demonstrate that dietary antioxidants can minimize the oxidative instability of proteins and lipids, and the protection may be linked to improved cellular antioxidant enzymatic activity.

Key words: broiler, oxidized oil, antioxidant-supplemented diet, meat quality, antioxidant enzyme activity

2014 Poultry Science 93:1561–1570
http://dx.doi.org/10.3382/ps.2013-03682

INTRODUCTION

Oxidation is a result of natural metabolic processes, but excessive formation of reactive species, such as free radicals, can damage important biomolecules (i.e., lipids, proteins, and nucleic acids) in the body of humans and animals alike. The rate of oxidation increases in result of the following: (1) high intake of oxidized lipids and prooxidants; (2) deterioration of sensitive polyunsaturated fatty acids (**PUFA**); and (3) low intake of antioxidative nutrients (Morrissey et al., 1998; Smet et al., 2008). In muscle foods, oxidative reactions continue postmortem and are a leading cause of quality deterioration during processing and storage. With a relatively high proportion of PUFA, poultry meat is more susceptible to oxidative processes, specifically lipid oxidation, than beef or pork. Therefore, incorporation of dietary antioxidants, such as vitamin E and Se in poultry feed, has been implemented to achieve optimal growth performance, reproduction, and meat quality.

A major challenge in broiler production is the expense of raising birds, where 60 to 75% of the total incurred cost is in feed alone (Tahir and Pesti, 2012). In recent years, the rising cost of raw materials and energy has driven up the prices of vitamin E and animal feedstuffs, forcing producers to find less expensive alternative dietary sources. Often, for economical poultry rearing, highly oxidized, recycled vegetable oils (e.g., reused frying oils) are often used as an added fat source in poultry feed to increase the energy density (Tavárez et al., 2011). Unfortunately, vegetable oils rich in PUFA are highly susceptible to oxidative deterioration. The products of lipid oxidation can decrease the nutrient content of the feed by reacting with proteins, lipids, and fat-soluble vitamins, which may even form toxic products that can adversely affect broiler performance

Received October 10, 2013.
Accepted February 8, 2014.

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and health (Engberg et al., 1996). Crespo and Esteve-Garcia (2002) reported that broilers deposit PUFA into their tissues similar to the rates present in their diet, and Eder et al. (2003) showed that dietary thermoxidized oils suppressed gene expression of lipogenic enzymes in rats. Hence, utilizing oxidized oil in broiler feed may result in decreased shelf-life and quality consistency of meat due to the potential suppression of gene expression for antioxidant enzymes.

Recently, nutrigenomic studies coupled with proteomic investigations have indicated a potential link between dietary nutrients and the expression of specific enzymes and metabolites in muscle (Hesketh, 2008). Li et al. (2009) reported that dietary supplementation with α-tocopherol improved meat tenderness and reduced lipid oxidation in broiler breast and thigh meat. However, the influence of dietary antioxidants on the genetic and regulatory mechanisms that define metabolic and physiological changes in muscle tissue is complex and poorly understood. Xiao et al. (2011) reported that EconomasE (**EcoE**), an algae-based antioxidant containing Se yeast, reduced the amount of vitamin E required in broiler feed without compromising growth performance or overall health. However, little work was done in determining the oxidative stability of breast meat quality during storage. The present study was designed to assess the influence of a natural algae-based Se yeast (EcoE) and organic mineral (Bioplex) antioxidant blend and oil quality on the oxidative and enzymatic properties of chicken broiler breast meat. To relate the study to in situ situations, harvested meat was packaged and stored in an oxygen-enriched (**HiOx**: 80% O_2/20% CO_2), air-permeable polyvinylchloride (**PVC**), or skin (**SK**) packaging systems during retail display at 2 to 4°C for up to 21 d.

MATERIALS AND METHODS

Materials

A commercial algae-based antioxidant, containing Se yeast as in EcoE and organic minerals as in Bioplex, was supplied by Alltech Inc. (Nicholasville, KY). Soybean oil was acquired from a local retailer, and the initial peroxide value (**POV**), determined according to AOCS (2007), was 23 mEq of O_2/kg. To create oxidized oil, aluminum pans (41 × 13 × 4 cm) each containing 5 kg of the above oil were heated in a convection oven at 95°C ± 5°C for up to 7 d. The POV of the oxidized oil was monitored intermittently. When the POV reached the target level (120 mEq of O_2/kg), heating was discontinued and the oxidized oil was cooled to room temperature. The final POV of the pooled oxidized oil was 121 mEq of O_2/kg, which was used immediately for diet preparation. All chemicals (reagent grade) were purchased from Fischer Scientific (Pittsburgh, PA) or Sigma-Aldrich (St. Louis, MO) unless specified otherwise.

Table 1. Ingredient and nutrient composition (as-fed basis) of the basal diet

Item	Starter diet (1–21 d)	Grower diet (22–42 d)
Ingredient (% of diet)		
Corn	53.41	61.26
Soybean meal (48% CP)	38.00	31.40
Soybean oil (low or high oxidized)	4.40	3.40
Salt	0.45	0.45
Limestone	1.33	1.30
Dicalcium phosphate	1.76	1.54
DL-Methionine	0.15	0.15
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
Nutrient (calculated value)		
AME_n (kcal/kg)	3.12	3.15
Protein, %	22.00	20.00
Ca (%)	1.00	0.90
Nonphytate P (%)	0.45	0.41
TSAA (%)	0.90	0.72
Lysine (%)	1.24	1.11

[1]Supplied per kilogram of diet for all diets: 11,025 IU of vitamin A (retinyl acetate), 0.0882 mg of vitamin D_3 (cholecalciferol), 0.91 mg of vitamin K_3 (2-methyl-1, 4-naphthoquinone), 2 mg of thiamine, 8 mg of riboflavin, 55 mg of niacin, 18 mg of Ca pantothenate, 5 mg of vitamin B_6 (pyridoxines), 0.221 mg of biotin, 1 mg of folic acid, 478 mg of choline, 28 μg of vitamin B_{12} (cyanocobalamin). Vitamin E (DL-α-tocopheryl acetate): 50 IU/kg for the basal diet with low oxidized oil (LO) and the basal diet with high oxidized oil (HO), 10 IU/kg for the basal diet with low oxidized oil, supplemented with antioxidants (ALO) and the basal diet with high oxidized oil, supplemented with antioxidants (AHO).

[2]Supplied per kilogram of diet for LO and HO diets: 80 mg of Fe as $FeSO_4 \cdot H_2O$, 90 mg of Mn as $MnSO_4 \cdot H_2O$, 125 mg of Cu as $CuSO_4 \cdot 5H_2O$, 100 mg of Zn as ZnO, 0.35 mg of I as KIO_3, and 0.30 mg of Se as sodium selenite. Supplied per kilogram of diet for ALO and AHO diets: 20 mg of Fe as Bioplex Fe (Alltech Inc., Nicholasville, KY), 23 mg of Mn as Bioplex Mn, 31 mg of Cu as Bioplex Cu, 25 mg of Zn as Bioplex Zn, 0.35 mg of iodine as KIO_3, and 0.30 mg of Se as Selplex.

Broiler Production

All procedures used in the study herein were approved by the University of Kentucky Animal Care and Use Committee. Three independent feeding trials (n = 3) over a 2-yr period were performed. In each, 960 male broilers were raised from 1 to 42 d of age and randomly placed in 48 floor pens with 20 birds per pen. Each pen was randomly designated 1 of 4 dietary treatments consisting of feeding (1) basal diet with low oxidized oil (**LO**); (2) basal diet with low oxidized oil, supplemented with antioxidants (**ALO**); (3) basal diet with high oxidized oil (**HO**); and (4) basal diet with high oxidized oil, supplemented with antioxidants (**AHO**). Broilers were randomly distributed into the 4 dietary groups with 12 replicate pens for each diet. Each pen was equipped with a feeder, a nipple drinker line, and a litter of soft wood shavings. Birds consumed feed (Table 1) in mash form and water on an ad libitum basis. A starter diet containing 22% CP and 3,120 kcal/kg was fed from 0 to 21 d of age and a grower diet containing 20% CP and 3,150 kcal/kg was fed from 21 to 42 d of age (Table 2). Photoperiod consisted of 22L:2D throughout the experiment.

Meat Preparation, Packaging, and Storage

After 42 d of feeding, one broiler from each of the 48 pens (4 diets × 12 pens) was randomly selected, humanely harvested, defeathered, and then chilled in ice slurries for 1.5 h. Both sides of the breast (pectoralis major) were then removed and skinned. Per diet, one randomly selected broiler breast was placed in a Cryovac black processor tray, CS977 (22 × 17 × 4 cm; Sealed Air Corporation, Elmwood Park, NJ) and sealed with Cryovac Lidstock 1050 MAP ethylene vinyl alcohol film (1.0 mil, <20 $cm^3/m^2/24$ h oxygen transmission rate at 4.4°C using an InPack Junior A10 packaging machine (Ross Industries Inc., Midland, VA). A gas mixture of 80% O_2/20% CO_2 (Scott-Gross Company Inc., Lexington, KY) was used for the HiOx packaging. For PVC, one breast per diet was placed on #2 supermarket white polystyrene trays (20.8 × 14.5 × 2.3 cm in dimension; Pactive LLC, Lake Forest, IL) and overwrapped with an air-permeable polyvinylchloride film (15,500–16,275 $cm^3/m^2/24$ h oxygen transmission rate at 23°C; E-Z Wrap Crystal Clear PVC Wrap, Koch Supplies, North Kansas City, MO). For SK, broiler breasts were packaged using Cryovac black processor trays and sealed with a Cryovac V834HB polyolefin film (4.0 mil, 1 $cm^3/m^2/24$ h oxygen transmission rate at 23°C).

Antioxidative Minerals and Vitamin in Muscle Tissue

Eight broilers per dietary treatment (total of 32 per trial) were humanely harvested, and the pectoralis major muscle tissue was removed and stored at –20°C until use. Selenium content was measured according to Olson et al. (1975) as detailed by Cantor and Tarino (1982). The Zn level was determined as described by Montaser and Golightly (1992). Vitamin E was determined using the procedure of Liu et al. (1996).

Lipid Oxidation

Lipid oxidation in stored muscle samples was measured as TBA reactive substances (**TBARS**) according to Sinnhuber and Yu (1977). The TBARS concentration, using a molar extinction coefficient of 152,000 *M*/cm for the chromophore, was expressed as milligrams of malondialdehyde (**MDA**) per kilogram of muscle.

Protein Oxidation

Because myofibrillar proteins are responsible for most of the meat quality attributes important to broilers [i.e., water-holding, tenderness, and texture (Xiong, 2000)], this muscle protein group was selected for protein oxidation analysis. Myofibrils were isolated from meat on the appropriate storage days using a rigor buffer containing 0.1 *M* KCl, 2 m*M* $MgCl_2$, 1 m*M* EGTA, and 10 m*M* K_2HPO_4 (pH 7.0) as previously described (Xiong, 2000). Protein concentration was determined by the Biuret method. Myofibril pellets were kept on ice, and all the measurements were completed within 24 h of isolation.

Protein carbonyls were measured according to the 2,4-dinitrophenylhydrazine colorimetric method as described by Levine et al. (1990). The carbonyl content expressed as nanomoles per milligram of protein was calculated using a molar absorption coefficient of 22,000 *M*/cm for the formed protein hydrazones. Sulfhydryls were determined using 5,5′ dithio-bis(2-nitrobenzoic acid; Ellman, 1959). Total sulfhydryl content was calculated using the molar extinction coefficient of 13,600 *M*/cm and expressed as nanomoles per milligram of protein.

Table 2. Composition of the experimental diets

	LO		ALO		HO		AHO	
Nutrient	Starter	Grower	Starter	Grower	Starter	Grower	Starter	Grower
ME (kcal/kg)	3,120	3,150	3,120	3,150	3,120	3,150	3,120	3,150
CP (%)	22	20	22	20	22	20	22	20
Lysine (%)	1.24	1.11	1.24	1.11	1.24	1.11	1.24	1.11
TSAA (%)	0.90	0.72	0.90	0.72	0.90	0.72	0.90	0.72
Available P (%)	0.45	0.41	0.45	0.41	0.45	0.41	0.45	0.41
Ca (%)	1.00	0.90	1.00	0.90	1.00	0.90	1.00	0.90
Vitamin E (IU/kg)	50	50	10	10	50	50	10	10
Se[1] (mg/kg)	0.30	0.30	–	–	0.30	0.30	–	–
Zn[2] (mg/kg)	100	100	25	25	100	100	25	25
Cu[2] (mg/kg)	125	125	31	31	125	125	31	31
Mn[2] (mg/kg)	90	90	23	23	90	90	23	23
Fe[2] (mg/kg)	80	80	20	20	80	80	20	20
EconomaseE[3] (mg/kg)	–	–	200	200	–	–	200	200
Soybean oil (%, low-oxidized)	4.4	3.4	4.4	3.4	–	–	–	–
Soybean oil (%, high-oxidized)	–	–	–	–	4.4	3.4	4.4	3.4

[1]As in Selenite (Alltech Inc., Nicholasville, KY).

[2]As inorganic minerals for the basal diet with low oxidized oil (LO) and the basal diet with high oxidized oil (HO); as in Bioplex for the basal diet with low oxidized oil, supplemented with antioxidants (ALO) and the basal diet with high oxidized oil, supplemented with antioxidants (AHO).

[3]A Se-yeast-based antioxidant blend (Alltech Inc.).

Antioxidant Enzymes

Six broilers per dietary treatment (total of 24 per trial) were humanely harvested. Immediately following exsanguination, aliquots of pectoralis major muscle samples (approximately 5 g each) were removed from each broiler, cryogenically frozen in liquid N_2 (–196°C), and stored in a –80°C freezer until use. Upon enzyme analysis, partially thawed muscle samples were mixed into 20 mL of chilled buffer (0.05 *M* Tris-HCl, pH 7.0) and homogenized for 30 s at 75,000 rpm with a model PT 10/35 Polytron homogenizer fitted with a PTA-20TS generator (Kinematica Ag, Luzern, Switzerland). The homogenate was centrifuged for 10 min at 10,000 × *g* at 4°C and the supernatant was filtered through 4 layers of grade 10 mesh cheese cloth. Protein content was measured using the Biuret method. The Cu-Zn superoxide dismutase (**SOD**) activity was determined according to Marklund and Marklund (1974) and Gatellier et al. (2004) using the inhibition of pyrogallol in a basic medium. Catalase (**CAT**) activity was measured by the rate of H_2O_2 disappearance according to Aebi (1974). Glutathione peroxidase (**GsPx**) activity was determined according to Beutler (1957).

Serum Protein Carbonyls

The chemical composition of blood serum, which is influenced by diet, is often used as an indicator of the nutritional status of an animal (Liotta et al., 2003). Hence, blood samples were taken from broilers by cardiac puncture immediately postmortem. Samples were allowed to clot at room temperature before centrifugation at 1,2000 × *g* for 15 min at room temperature to separate serum. The serum was transferred to Nalgene tubes, cryogenically frozen in liquid nitrogen, then stored in an –80°C freezer. Serum was thawed at 4°C in darkness, then protein carbonyl content was determined according to Levine et al. (1990) as stated above.

Statistical Analysis

Three independent bird feeding trials (n = 3) over a 2-yr period each with duplicate or triplicate muscle sample analyses were conducted. Data were subjected to ANOVA using the Statistix software 9.0 (Analytical Software, Tallahassee, FL) with the GLM procedure to determine the significance of main treatment factors (diet, packaging systems, and storage time). Least significant difference all-pairwise multiple comparisons were performed to separate the means when a treatment effect was found to be significant ($P < 0.05$).

RESULTS AND DISCUSSION

Tissue Antioxidative Minerals and Vitamins

Minerals and vitamins are generally added to animal feedstuff for growth, maintenance, and sustenance of life; yet the bioavailability, absorption, and distribution of these micronutrients in various tissues may vary with feed quality. Breast meat was chosen for the analysis because it is the most valuable cut on the bird and a greater distribution of antioxidative minerals and vitamins to this area may help improve oxidative stability. The effects of dietary antioxidant supplementation and oil quality on Se, Zn, and vitamin E content in broiler breast tissue can be seen in Table 3. Diets with antioxidant supplementation (ALO, AHO) significantly ($P < 0.05$) increased tissue Se content compared with the basal diet (LO, HO). There was no significant ($P < 0.05$) difference in tissue Zn and vitamin E levels among dietary treatments. However, birds fed a high-oxidized diet had slightly lower Zn and vitamin E levels compared with their respective counterparts. Boler et al. (2012) reported lower vitamin E levels in the back fat of barrows fed a high-oxidized corn oil diet supplemented with synthetic antioxidants compared with the fresh corn oil diet supplemented with synthetic antioxidants.

Lipid Oxidation

For all dietary treatments, lipid oxidation increased throughout the first 7 d of storage under each packaging condition (Table 4). The HiOx and PVC meat samples exhibited significant signs of microbial spoilage after 14 and 7 d, respectively, and therefore were not analyzed beyond these storage times. Compared with the HO dietary treatments, regardless of antioxidant supplementation, the low-oxidized samples (LO, ALO) had lower TBARS values in all packaging conditions, in agreement with Tavárez et al. (2011) who reported reduced TBARS production in retail display breast meat from broilers fed a commercial blend of ethoxyquin and propyl gallate. On d 14, the TBARS value of the HO dietary group was significantly higher ($P < 0.05$) compared with the LO samples packaged under HiOx. Furthermore, HiOx and PVC produced higher amounts of TBARS ($P < 0.05$) than SK throughout storage. Delles et al. (2011) reported similar oxidative susceptibility and subsequent higher TBARS values of pork muscle packaged in HiOx and PVC compared with vacuum packaging (similar to SK). Samples from birds fed an antioxidant-supplemented diet, regardless of oil quality, showed lower TBARS formation compared with basal dietary regimens. Other studies focusing on natural antioxidants have also shown that feeding broilers high levels of α-tocopherol (De Winne and Dirinck, 1996) and Se (Ryu et al., 2005) delays the onset of oxidative off-flavor formation in chicken meat during storage.

Lipid oxidation in muscle foods occurs primarily in the highly unsaturated phospholipids of the subcellular membranes (Frankel, 1980). The lower levels of TBARS formation in the antioxidant-supplemented diets, regardless of oil quality, may be attributed to EcoE. The algae-Se yeast based antioxidant contains natural

Table 3. Effects of dietary antioxidants and oil quality on tissue vitamin and mineral content of broilers

Diet[1]	Se (μg/mg)	Zn (mg/kg)	Vitamin E (μg/g)
LO	170.78 (26.5)[b]	6.85 (0.8)	134.18 (20.9)
ALO	300.68 (73.5)[a]	6.67 (0.8)	128.98 (20.3)
HO	172.10 (25.3)[b]	6.58 (0.7)	127.36 (13.8)
AHO	328.94 (87.3)[a]	6.37 (0.7)	121.13 (18.7)
P-value	<0.0001	0.3449	0.4891

[a,b]Means (n = 3) between dietary treatments without a common lowercase superscript differ significantly ($P < 0.05$).

[1]Basal diet with low oxidized oil (LO); basal diet with high oxidized oil (HO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO).

sources of radical-scavenging lipid soluble compounds such as carotenoids and polyphenols (Kobayashi and Sakamoto, 1999; Wang et al., 2009). The molecular structures of these compounds allow them to protect highly oxidizable PUFA through neutralization of free radicals in the cellular and subcellular membranes. In addition, Se is an essential trace mineral that serves as a key component (co-factor) in various selenoproteins and selenoenzymes (Battin and Brumaghim, 2009). The greater amount of Se present in the tissue of broilers fed an antioxidant-supplemented diet (ALO or AHO) would conceivably promote glutathione peroxidase activity, in agreement with Mahan et al. (1999) who reported that pigs fed a diet containing organic Se-enriched yeast had higher serum Se concentrations and greater serum glutathione peroxidase activity. Furthermore, tissue vitamin E or Zn levels did not significantly differ between diets (Table 3), indicating that an algae-based Se yeast and organic mineral antioxidant blend can be an effective vitamin E and Zn replacement.

Protein Oxidation

Dietary intake of oxidized oil has been reported to increase the oxidative stress in vivo and potentially cause an imbalance between the production of reactive oxygen species (**ROS**) and the defense mechanism of an animal's body (Hayam et al., 1995; Boler et al., 2012). Furthermore, oxidized oil may damage dietary vitamins and increase the susceptibility of the gastrointestinal tract and other tissues to lipid and protein oxidation (Sheehy et al., 1994; Zhang et al., 2011b). Proteins are a major target of ROS; the accumulation of oxidized products in the muscle tissue leads to meat quality deterioration. Hence, oxidative chemical modifications, including reduced tryptophan fluorescence, loss of sulfhydryl groups, intra- and intermolecular crosslinks, and formation of carbonyl derivatives, have a detrimental effect on meat quality (Xiong, 2000).

In the present study, muscle tissue protein carbonyl content increased during storage for all dietary treatments and all packaging conditions (Table 5). The carbonyl level in HO samples, irrespective of packaging condition, was higher than those in LO samples. However, muscle samples from antioxidant-supplemented diets had lower carbonyl content compared with the basal group. For example, SK samples after 21 d had lower amounts of carbonyls ($P < 0.05$) in the ALO group compared with LO. The effect of packaging systems and storage time on protein carbonyl formation was overall similar to that of TBARS, suggesting a possible relationship between lipid oxidation and protein carbonyl formation. Malondialdehyde, a secondary dicarbonyl product of lipid oxidation, can interact with amine groups in proteins, generating protein-bound carbonyls (Buttkus, 1967; Hidalgo et al., 1998). The loss of TBARS formation after 7 d and, conversely, the

Table 4. Effects of diets on lipid oxidation (TBA reactive substances, mg/kg of malondialdehyde) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2°C

		Dietary treatment[1]				
Packaging	Day	LO	ALO	HO	AHO	Pkg
	0	0.046 ± 0.014[c]	0.064 ± 0.014[c]	0.05 ± 0.009[c]	0.053 ± 0.010[c]	
HiOx	4	0.172 ± 0.039[b]	0.136 ± 0.029[b]	0.208 ± 0.041[b]	0.165 ± 0.037[b]	*
	7	0.315 ± 0.048[a,AB]	0.279 ± 0.033[a,B]	0.379 ± 0.023[a,A]	0.348 ± 0.023[a,AB]	*
	14	0.305 ± 0.033[a,B]	0.269 ± 0.032[a,B]	0.399 ± 0.043[a,A]	0.342 ± 0.032[a,AB]	*
PVC	4	0.212 ± 0.036[b]	0.177 ± 0.028[b]	0.254 ± 0.029[b]	0.230 ± 0.034[b]	*
	7	0.278 ± 0.05[a,B]	0.232 ± 0.028[a,B]	0.352 ± 0.018[a,A]	0.288 ± 0.021[a,AB]	*
SK	4	0.116 ± 0.025[b]	0.116 ± 0.031[b]	0.158 ± 0.028[b]	0.133 ± 0.031[b]	*
	7	0.262 ± 0.063[a]	0.229 ± 0.054[a]	0.298 ± 0.07[a]	0.267 ± 0.063[a]	*
	14	0.242 ± 0.041[a]	0.214 ± 0.031[a]	0.277 ± 0.033[a]	0.269 ± 0.038[a]	*
	21	0.247 ± 0.012[a,AB]	0.204 ± 0.036[a,B]	0.279 ± 0.012[a,A]	0.256 ± 0.039[a,AB]	

[a–c]Means (n = 3) between days within the same diet (same column) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A,B]Means (n = 3) between diets on the same day (same row) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

[1]Basal diet with low oxidized oil (LO); basal diet with high oxidized oil (HO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO).

*Means between packaging systems (Pkg) on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

Table 5. Effects of diets on protein carbonyl formation (nmol/mg of protein) in broiler meat packaged in oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2°C

		Dietary treatment[1]				
Packaging	Day	LO	ALO	HO	AHO	Pkg
	0	$0.189 \pm 0.032^{d,B}$	$0.167 \pm 0.025^{c,B}$	$0.235 \pm 0.024^{c,A}$	$0.203 \pm 0.022^{d,AB}$	
HiOx	4	$0.565 \pm 0.079^{c,AB}$	$0.472 \pm 0.065^{b,B}$	$0.707 \pm 0.013^{b,A}$	$0.608 \pm 0.100^{c,AB}$	*
	7	1.004 ± 0.084^{b}	0.994 ± 0.130^{a}	1.050 ± 0.059^{a}	0.927 ± 0.068^{b}	*
	14	1.14 ± 0.115^{a}	1.009 ± 0.147^{a}	1.11 ± 0.232^{a}	1.127 ± 0.165^{a}	*
PVC	4	$0.508 \pm 0.083^{b,AB}$	$0.417 \pm 0.064^{b,B}$	$0.625 \pm 0.117^{b,A}$	$0.538 \pm 0.093^{b,AB}$	*
	7	$0.925 \pm 0.051^{a,B}$	$0.844 \pm 0.035^{a,B}$	$1.065 \pm 0.079^{a,A}$	$0.949 \pm 0.056^{a,AB}$	*
SK	4	$0.387 \pm 0.052^{c,B}$	$0.314 \pm 0.068^{c,C}$	$0.460 \pm 0.074^{c,A}$	$0.410 \pm 0.061^{c,AB}$	*
	7	$0.751 \pm 0.015^{b,BC}$	$0.674 \pm 0.033^{b,C}$	$0.842 \pm 0.043^{b,A}$	$0.792 \pm 0.060^{b,AB}$	*
	14	0.920 ± 0.155^{a}	0.934 ± 0.109^{a}	0.945 ± 0.164^{b}	0.968 ± 0.129^{a}	*
	21	$1.025 \pm 0.110^{a,B}$	$0.947 \pm 0.084^{a,C}$	$1.092 \pm 0.084^{a,A}$	$1.046 \pm 0.086^{a,AB}$	

[a–d]Means (n = 3) between days within the same diet (same column) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

[A–C]Means (n = 3) between diets on the same day (same row) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

[1]Basal diet with low oxidized oil (LO); basal diet with high oxidized oil (HO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO).

*Means between packaging systems (Pkg) on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

increase in protein carbonyl content, suggests that some of the MDA bound to amino acid side chains forming extra carbonyl compounds.

Sulfhydryls from cysteine residues are highly susceptible to oxidation by most forms of ROS and provide an additional assessment of protein oxidation (Lund et al., 2011). As shown in Table 6, samples from broilers fed an antioxidant treatment (ALO, AHO), regardless of oil quality or packaging condition, showed greater protein sulfhydryl retention compared with their respective controls (LO, HO). Furthermore, samples from broilers fed a HO diet initially (d 0) already had a lower ($P < 0.05$) sulfhydryl content compared with LO samples; this oxidation-associated effect was extended to stored meat packaged under all 3 conditions [i.e., 14 d for HiOx; 4 d for PVC; and 4, 7, and 21 d for SK (Table 6)]. Significant ($P < 0.05$) losses of sulfhydryls occurred from d 0 to 4 in HiOx and PVC for all dietary treatments, whereas SK samples remained relatively constant. By d 7, all packaging systems showed significant ($P < 0.05$) losses in protein sulfhydryls. The reduced carbonyl formation and sulfhydryl disappearance in antioxidant-supplemented samples may be due to reduced reactive species formation in vivo. The EcoE functions as a natural antioxidant by altering the expression of various gene transcripts, which may increase the total antioxidant capacity in the serum of broilers (Xiao et al., 2011). Selenium, an antioxidant mineral, has strong antioxidant properties and serves as a cofactor for glutathione peroxidase, an enzyme that catalyzes the reduction of hydrogen peroxide and lipid peroxides, thereby preventing oxidative damage. Finally, various studies have reported beneficial effects of organic minerals on broiler performance, health, and meat quality that may be attributed to its greater bioavailability and absorption compared with inorganic minerals (Castellini et al., 2002; Bao et al., 2007; Aksu et al., 2011).

Serum Protein Carbonyls

Dietary PUFA greatly influence serum lipid concentrations, lipid profiles, and lipid metabolism by regulating gene expression through alteration of transcription factors involved in absorption, extracellular transport, cellular uptake, metabolism, and elimination of lipids in the animal (Jump, 2002; Ringseis and Eder, 2005). Dietary supplementation with oxidized oil resulted in higher levels of protein carbonyl formation in blood serum (Figure 1), in agreement with Zhang et al. (2011b), who reported that 5% inclusion of oxidized animal-vegetable fat in broiler diets increased plasma carbonyl content. Low-oxidized samples supplemented with antioxidants (ALO) showed slightly lower ($P = 0.06$) serum carbonyl content compared with all other dietary groups. The greater serum carbonyl content in broilers fed a HO diet may be attributed to the propagation of dietary lipid peroxides and subsequent attack of blood serum proteins, such as albumin, upon the transportation of fatty acids by chylomicrons to various tissues in the bird. Hence, feeding broilers a high-oxidized diet can increase oxidative stress in vivo.

Antioxidant Enzyme Activities

Oxidative stress is the imbalance between the production and degradation of ROS, such as superoxide anion, hydrogen peroxide, and lipid peroxides. Enzymatic inactivation of ROS in muscle tissue is mainly achieved by SOD, CAT, and GsPx with each having a unique mechanism. The SOD and CAT are antioxidant enzymes that directly react with radical species, whereas GsPx regenerates oxidized antioxidants. Although the metabolic pathways for oxidized lipids after ingestion has not been fully elucidated, some studies have suggested that absorption of lipid peroxides is depen-

Table 6. Effects of diets on free sulfhydryl (nmol/mg of protein) in broiler meat packaged oxygen-enriched (HiOx), air-permeable polyvinylchloride (PVC), or skin (SK) packaging systems during refrigerated storage at 2°C

		Dietary treatment[1]				
Packaging	Day	LO	ALO	HO	AHO	Pkg
	0	87.28 ± 2.2^{a,B}	93.48 ± 2.5^{a,A}	81.16 ± 2.9^{a,C}	82.41 ± 3.0^{a,C}	
HiOx	4	70.23 ± 5.7^{b,BC}	77.49 ± 3.9^{b,A}	65.00 ± 6.0^{b,C}	72.93 ± 1.9^{b,AB}	*
	7	52.95 ± 2.6^{c}	52.24 ± 5.1^{c}	50.46 ± 2.0^{c}	55.92 ± 2.9^{c}	*
	14	45.72 ± 1.3^{d,A}	40.43 ± 3.7^{d,AB}	38.08 ± 2.1^{d,B}	37.81 ± 3.1^{d,B}	*
PVC	4	66.12 ± 6.4^{b,B}	71.54 ± 5.2^{b,A}	61.81 ± 4.4^{b,B}	63.76 ± 4.2^{b,B}	*
	7	51.54 ± 1.8^{c,B}	59.09 ± 2.2^{c,A}	44.36 ± 0.5^{c,C}	52.27 ± 3.1^{c,B}	*
SK	4	83.75 ± 1.6^{a,A}	85.36 ± 2.1^{b,A}	76.34 ± 3.6^{a,C}	79.37 ± 3.2^{a,B}	*
	7	70.72 ± 3.6^{b,A}	70.23 ± 6.6^{c,A}	58.44 ± 6.7^{b,B}	64.81 ± 4.4^{b,AB}	*
	14	52.28 ± 8.1^{c}	58.08 ± 7.5^{d}	56.79 ± 5.3^{b}	57.98 ± 6.4^{c}	*
	21	48.99 ± 3.4^{c,A}	53.44 ± 4.2^{d,A}	39.87 ± 2.1^{c,B}	41.66 ± 4.9^{d,B}	

$^{a–d}$Means (n = 3) between days within the same diet (same column) within the same packaging system without a common lowercase superscript differ significantly ($P < 0.05$).

$^{A–C}$Means (n = 3) between diets on the same day (same row) within the same packaging system without a common uppercase superscript differ significantly ($P < 0.05$).

[1]Basal diet with low oxidized oil (LO); basal diet with high oxidized oil (HO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO).

*Means between packaging systems (Pkg) on the same days differ significantly ($P < 0.05$); there was no diet × packaging interaction.

dent upon antioxidant defense enzymes that metabolize lipid derivatives in the mucosa of the digestive tract (Aw et al., 1992) and in the liver (Takahashi et al., 2002; Zalejska-Fiolka et al., 2010).

Ingestion of the high-oxidized diets (HO, AHO) resulted in reduction in the activity of SOD, CAT, and GsPx compared with their respective low-oxidized counterparts (Figure 2). Specifically, SOD activity was significantly ($P < 0.05$) higher in samples from antioxidant-supplemented diets than the basal diet. Gatellier et al. (2004) reported an increase in tissue SOD activity from bovine fed a high vitamin E pasture diet. Interestingly, the presence of antioxidants in the high-oxidized diet alleviated some of the negative effects of oxidized oil on SOD, resulting in similar SOD enzymatic rates between LO and AHO samples, which may be attributed to the gene upper regulation in response to antioxidant supplementation. There was no significant correlation between SOD and CAT activity (Figure 2) and Zn and vitamin E levels (Table 3). Tissue samples from broilers fed a high-oxidized treatment without antioxidant supplementation (HO) showed the lowest ($P < 0.05$) SOD activity. The CAT showed a similar trend to that of SOD. In agreement, Srivastava et al. (2010) noticed a significant decline in tissue SOD and CAT activity in rats fed repeatedly boiled sunflower oil. The antioxidant treatment groups ALO and AHO had higher CAT reactivity compared with LO and HO, respectively. Prolonged dietary stress may cause oxidative tissue damage as evidenced by loss in the activity of the coupled antioxidant enzymes, SOD and CAT. DaCosta and Huang (2007) reported a general decline in SOD and CAT antioxidant enzyme activities and an increase in lipid oxidation of grass plant species upon drought stress, indicating that the production of free radicals may exceed the scavenging capacity of the antioxidant defense system under extreme stress conditions.

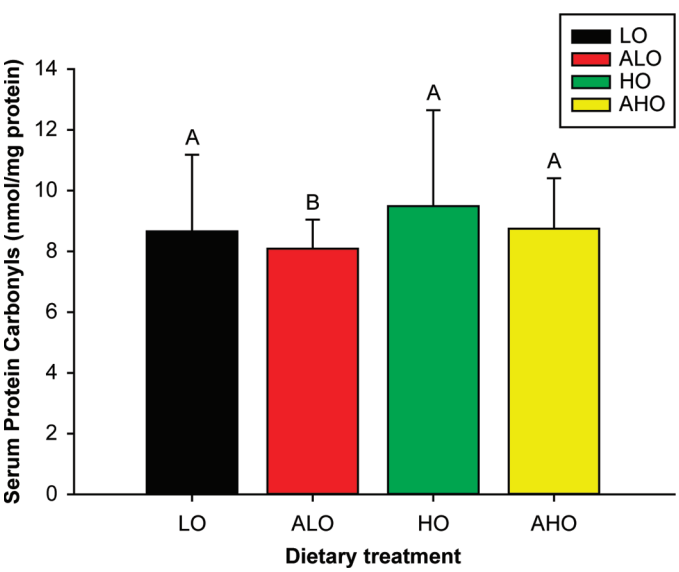

Figure 1. Effects of dietary treatments on carbonyl content isolated from blood. Basal diet with low oxidized oil (LO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); basal diet with high oxidized oil (HO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO). Different uppercase letters (A,B) indicate difference ($P = 0.06$) of means (n = 3) between dietary treatments. Color version available in the online PDF.

Glutathione peroxidase is a key antioxidant enzyme within most cells that reduces hydrogen peroxide to water and lipid peroxides to their respective alcohols (Sies, 1999). Compared with basal dietary regimens, regardless of oil quality, the antioxidant treatment groups (ALO, AHO) had significantly ($P < 0.05$) higher GsPx activity compared with the basal dietary group (LO, HO). The HO had the lowest GsPx activity. Bansal et al. (2005) reported a decrease in glutathione reductase activity as well as total glutathione content in the liver of rats fed nitrosamine compounds, a hepatic carcino-

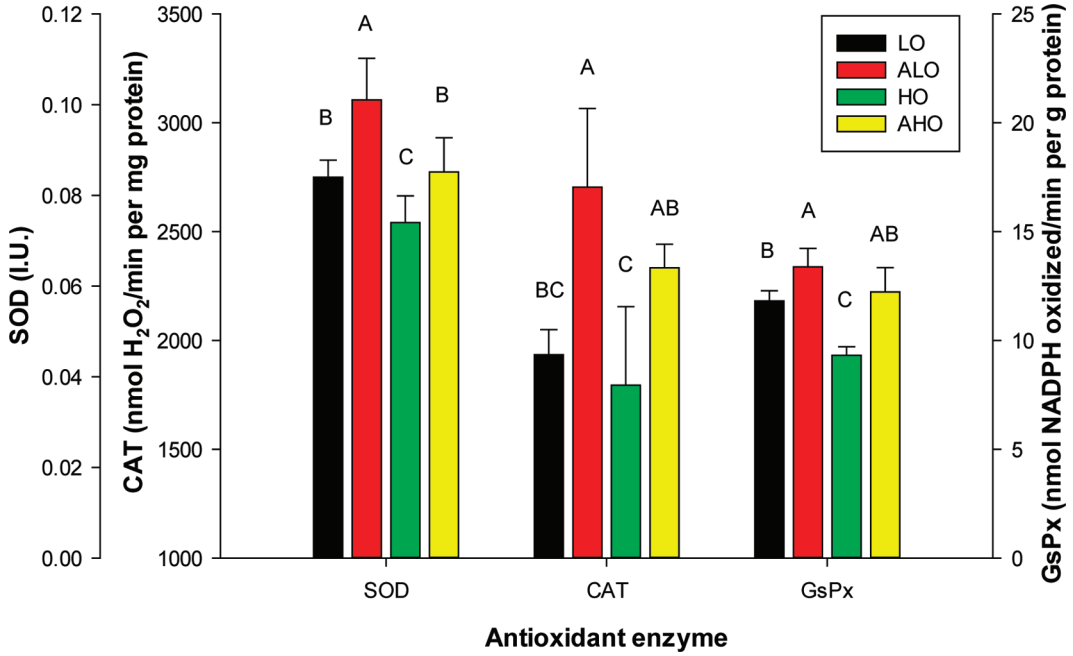

Figure 2. Effects of dietary treatments on tissue antioxidant enzyme activities (superoxide dismutase, SOD; catalase, CAT; glutathione peroxidase, GsPx) in broiler meat. Basal diet with low oxidized oil (LO); basal diet with low oxidized oil, supplemented with antioxidants (ALO); basal diet with high oxidized oil (HO); and basal diet with high oxidized oil, supplemented with antioxidants (AHO). Different uppercase letters (A–C) indicate significant difference ($P < 0.05$) of means (n = 3) between dietary treatments. Color version available in the online PDF.

gen. However, rats pretreated with oral doses of vitamin E showed improved glutathione reductase activity and increased levels of total glutathione content, indicating an elevation in antioxidant activity and a counteraction against nitrosamine-induced oxidative stress. As shown in Table 3, birds fed diets supplemented with the algae-based Se yeast and organic mineral antioxidant blend had higher levels of tissue Se, coinciding with a higher reactivity of GsPx (Figure 2). Zhang et al. (2011a) also found that oral doses of Se increased blood and serum GsPx activity.

The reduction of the tissue antioxidant enzyme efficacy as a consequence of feeding oxidized oil may be attributed to chemical toxicity of oxidized PUFA. It appears that the attenuated enzyme activity may be ultimately related to the alteration or reduction in gene expression and transcription. Ringseis and Eder (2005) evidenced reduced transcription of proteosomal and lysosomal enzymes in rats fed oxidized cholesterol. The proteosomal pathway is responsible for the protein repair mechanism required to rescue oxidized proteins and prevent cellular cytoxicity. Hence, a loss in proteasome function may contribute to the accumulation of oxidized proteins and loss in the activity of the antioxidant defense mechanism (Grune et al., 2003) and ultimately reduce meat quality. It appears that the observed differences between dietary treatments (LO, ALO, HO, AHO) in muscle tissue TBARS, protein carbonyls, and sulfhydryls were associated with CAT, SOD, and GsPx antioxidant enzyme activity. Figure 3 depicts a schematic diagram of the potential interaction of oxidized oils with respect to broiler meat oxidative stability and antioxidant enzyme potential. Nevertheless, more work is needed to fully clarify the mechanism of dietary antioxidants and oxidized oil on the metabolic and physiological changes in muscle tissue.

Conclusion

The results indicate that feeding diets with high-oxidized oil increased the vulnerability of lipids and proteins to oxidation and reduced the activities of tissue antioxidant defense enzymes. However, the dietary supplementation with an algae-based Se yeast and organic mineral antioxidant blend negated these effects. Furthermore, dietary antioxidant supplementation imparted a protective barrier against oxidation of broiler breast meat under HiOx, PVC, and SK packaging conditions throughout retail display. The improved oxidative stability appears to be associated with enhanced cellular antioxidant enzymatic activity and reduced ROS propagation in vivo. Further research is warranted to establish the precise in situ relationship between dietary antioxidants, tissue enzyme activity, and meat quality.

ACKNOWLEDGMENTS

The authors thank the animal caretakers of the Center for Applied Nutrigenomics and Applied Animal Nutrition for their assistance. This project was a collaborative study of the Alltech–University of Kentucky Nutrition Research Alliance. Publication as journal article number 13-07-119 of the Kentucky Agricultural Experiment Station, Lexington.

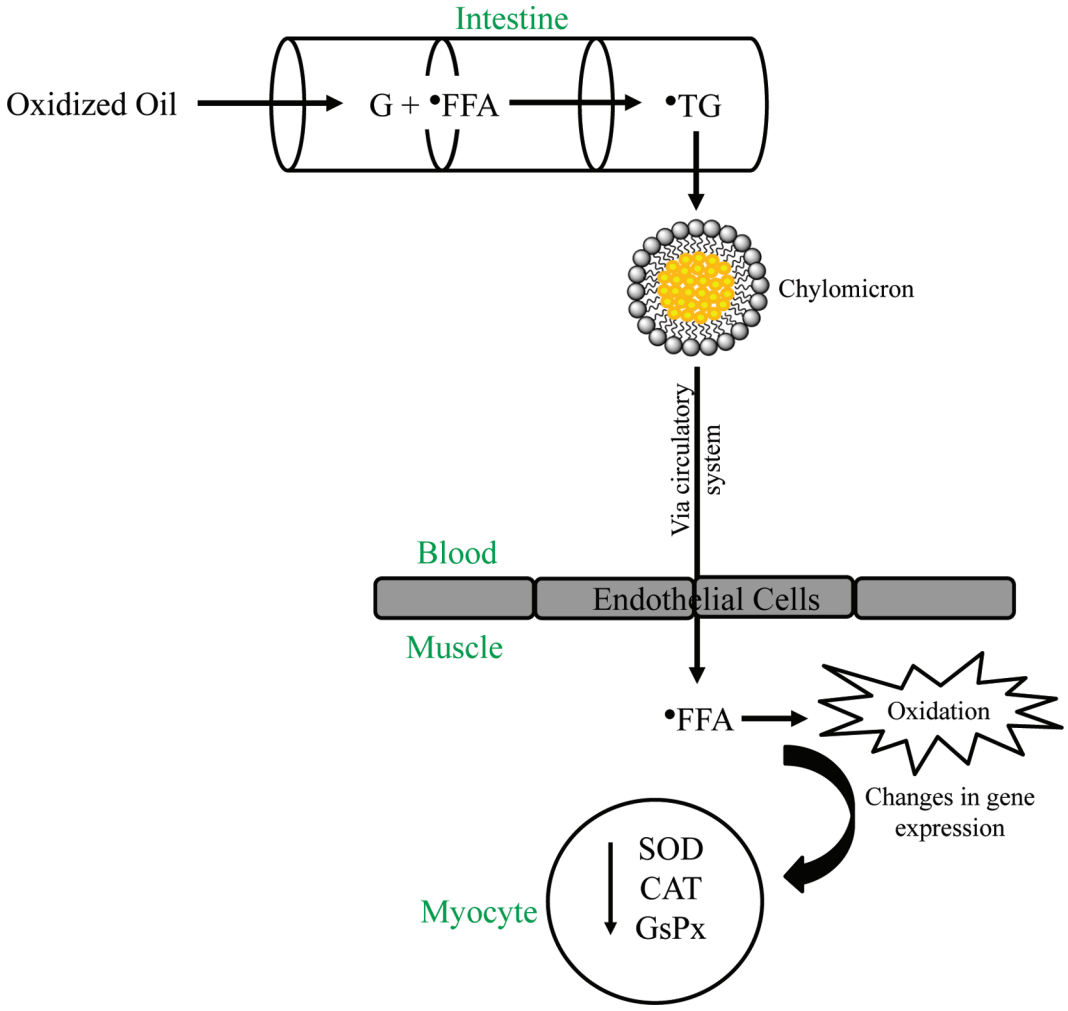

Figure 3. Schematic diagram of the mechanism of dietary oxidized oil on muscle tissue oxidation. G: glycerol; FFA: free fatty acid; TG: triacylglycerol; SOD: superoxide dismutase; CAT: catalase; GsPx: glutathione peroxidase. Color version available in the online PDF.

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