Supplementation of fats and oils in commercial broiler diets has become routine to achieve the recommended energy requirement, and realize optimum growth and feed efficiency. Vegetable oils, which are rich in unsaturated fatty acids, are particularly susceptible to oxidation. The oxidation process involves the generation of free radicals, which may then react with molecular oxygen to produce peroxide free radicals and lipid peroxidation (Sherwin, 1978; Fellenberg and Speisky, 2006). Oxidation products can negatively affect energy and lipid soluble nutrient values in the diet (Sherwin, 1978), be cytotoxic to the body (Esterbauer, 1993), and impair broiler health and growth (Engberg et al., 1996). In addition, these oxidants induced oxidative stress in broilers and negatively affected fresh meat quality traits, especially in flavor, color, drip loss, texture, and nutritive value (Gray et al., 1996; Lauridsen et al., 1999). A diet high in polyunsaturated fatty acids (PUFA) tends to increase the potential for lipid peroxidation and reduce the antioxidative capability of broilers (Jung et al., 2010).

To prevent oxidative deterioration of dietary components, synthetic antioxidants such as ethoxyquin and propyl gallate are added to the feed for their effective control of dietary lipid oxidation (Madhavi and Salunkhe, 1995). In addition, ethoxyquin improves growth performance (Cabel et al., 1988; Dibner et al., 1996; Wang et al., 1997) and stabilizes lipids in meat when supplemented in the diet (Bartov and Bornstein, 1972, 1981; Webb et al., 1978). Propyl gallate is a polyphenol widely used to chelate iron ions, which are a catalyst in the oxidation process (Madhavi and Salunkhe, 1995), and to improve stability of vegetable oils under storage (Pinkowski et al., 1986; Hawrysh et al., 1992). The combination of both had growth performance benefits in broilers (Tavarez et al., 2011). The objective of this study was to evaluate the effect of the antioxidant effects of a dietary antioxidant blend and vitamin E on growth performance, oxidative status, and meat quality in broiler chickens fed a diet high in oxidants

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ABSTRACT The aim of the study was to determine the effects of a dietary antioxidant blend (AB) and vitamin E on performance, oxidative status, and meat quality. Cobb 500 male broilers (n = 1,200, d 0) were randomly distributed into 6 treatments with 10 replicate pens. Treatments included 1) HO: high oxidant diet, vitamin E at 10 IU/kg, 3% oxidized soybean oil, 3% polyunsaturated fatty acid (PUFA) source; 2) VE: the HO diet with vitamin E at 200 IU/kg; 3) AOX: the HO diet with AB at 135 mg/kg; 4) VE+AOX: the HO diet with vitamin E at 200 IU/kg and AB at 135 mg/kg; 5) SC: standard control; and 6) PC: positive control, the SC diet with AB at 135 mg/kg. From d 0 through d 21, high oxidant diet treatment birds had greater BW, ADFI than the SC birds; the AOX birds had better G:F on d 10 and 42, and from d 0 to 42 than SC birds (P < 0.05). The plasma TBA reactive substance level was lower in the AOX birds than the VE treatment birds in all phases (P < 0.05). High oxidant diet treatment birds had greater α-1-acid glycoprotein levels on d 10 than SC and PC birds (P < 0.05). The AOX, PC, and SC birds had a greater level of uric acid than the HO and VE+AOX birds on d 10. Superoxide dismutase expression in the liver was less with the HO treatment compared with the SC treatment on d 7 (P < 0.05). The vitamin E concentration in the breast muscle was greatest in the VE birds, whereas vitamin A concentration was greater in the PC birds compared with the SC birds on d 21 (P < 0.05). Compared with VE and AOX, the HO treatment had greater drip loss (P < 0.05). In conclusion, dietary addition of AOX was effective in improving growth, moderately restored the whole body antioxidant capability, and reduced drip loss.

Key words: broiler chicken, antioxidant, performance, meat quality, oxidative status

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INTRODUCTION

Supplementation of fats and oils in commercial broiler diets has become routine to achieve the recommended energy requirement, and realize optimum growth and feed efficiency. Vegetable oils, which are rich in unsaturated fatty acids, are particularly susceptible to oxidation. The oxidation process involves the generation of free radicals, which may then react with molecular oxygen to produce peroxide free radicals and lipid peroxidation (Sherwin, 1978; Fellenberg and Speisky, 2006). Oxidation products can negatively affect energy and lipid soluble nutrient values in the diet (Sherwin, 1978), be cytotoxic to the body (Esterbauer, 1993), and impair broiler health and growth (Engberg et al., 1996). In addition, these oxidants induced oxidative stress in broilers and negatively affected fresh meat quality traits, especially in flavor, color, drip loss, texture, and nutritive value (Gray et al., 1996; Lauridsen et al., 1999). A diet high in polyunsaturated fatty acids (PUFA) tends to increase the potential for lipid peroxidation and reduce the antioxidative capability of broilers (Jung et al., 2010).

To prevent oxidative deterioration of dietary components, synthetic antioxidants such as ethoxyquin and propyl gallate are added to the feed for their effective control of dietary lipid oxidation (Madhavi and Salunkhe, 1995). In addition, ethoxyquin improves growth performance (Cabel et al., 1988; Dibner et al., 1996; Wang et al., 1997) and stabilizes lipids in meat when supplemented in the diet (Bartov and Bornstein, 1972, 1981; Webb et al., 1978). Propyl gallate is a polyphenol widely used to chelate iron ions, which are a catalyst in the oxidation process (Madhavi and Salunkhe, 1995), and to improve stability of vegetable oils under storage (Pinkowski et al., 1986; Hawrysh et al., 1992). The combination of both had growth performance benefits in broilers (Tavarez et al., 2011). The objective of this study was to evaluate the effect of the antioxidant...
blend (AB: ethoxyquin and propyl gallate) and vitamin E, a nutrient and antioxidant, on growth performance, oxidative status, and meat quality in broilers fed diets high in oxidants.

**MATERIALS AND METHODS**

**Birds and Experimental Design**

The complete protocol was reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee. Cobb 500 male broilers (n = 1,200, 44.7 ± 0.8 g) were acquired at day of hatch (Cobb-Vantress Inc., Wadesboro, NC) and placed in group pens in an environmentally controlled facility at the Virginia Tech Turkey Center (Blacksburg). Chickens were randomly distributed into 60 floor pens to balance initial weight across treatments and were assigned to 6 treatments with 10 replicate pens each and 20 birds per pen. Treatments included 1) HO: high oxidant diet with vitamin E at 10 IU/kg (NRC, 1994), 3% oxidized oil, 3% PUFA source [containing docosahexaenoic acid (DHA) at 41.2%]; 2) VE: the HO diet with vitamin E at 200 IU/kg; 3) AOX: the HO diet with AB at 135 mg/kg, 4) VE+AOX: the HO diet with both vitamin E at 10 IU/kg, 3% nonoxidized soy oil, no PUFA source; and 5) SC: standard control, a corn-soy diet with vitamin E at 10 IU/kg, 3% nonoxidized soy oil, no PUFA source; and 6) PC: positive control, the SC diet with AB at 135 mg/kg. Treatment and control diet information, and diet composition are summarized in Tables 1 and 2.

The antioxidant blend (Agrado Plus), PUFA source (Trevera) and oxidized oil were provided by Novus International Inc. (St. Charles, MO). To prepare the oxidized oil, soybean oil was heated to 95°C to reach peroxide value of approximately 180 mEq/kg. Oxidized oil was heated to 95°C and oxidized by continuously bubbling air at a rate of 80 L/min for up to 72 h. Peroxide values were determined hourly according to AOCS (2007) methods (peroxide value acetone/chloroform method, Cd853) to reach peroxide value of approximately 180 mEq/kg of oil.

Formulations were adjusted according to phase feeding practices as chickens advanced in age and weight as recommended by the breeder (Cobb-500, 2012): starter (d 0 to 10), grower (d 11 to 21), and finisher (d 23 to 42). The broiler grower house had negative pressure ventilation with side wall inlets and fans, circulation fans, and end wall fans with a Choretme Choretronics environment control system (Milford, IN). Pens were 1.22 × 2.44 m in dimension on concrete floors bedded with fresh wood shavings. Each pen was equipped with a nipple drinker line and galvanized tube feeder. Broilers were weighed by pen, and feed consumption determined on d 10, 21, and 42.

**Sample Acquisition and Preparation**

Blood samples were collected by brachial venipuncture into 4-mL heparin vacuum tubes from one tagged chicken at the end of each phase. On d 7, 14, and 21, one bird per pen (n = 10 per treatment) was randomly selected for euthanasia by cervical dislocation to provide tissue samples. The central lobe of the liver from each chicken was flash frozen in liquid nitrogen, and then stored along with plasma samples at −80°C until assays were conducted. On d 42, one chicken from each pen was harvested in the processing facility for samples and carcass data collection.

**Plasma TBA Reactive Substances, Alpha-1-Acid Glycoprotein, and Uric Acid**

Thiobarbituric acid reactive substances (TBARS) were determined using a commercial kit from Cayman Chemical Company (Ann Arbor, MI). To determine the concentrations of uric acid and α-1-acid glycoprotein (AGP) in the plasma, commercial kits were acquired from Teco Diagnostics (Anaheim, CA) and Life Diagnostics (West Chester, PA), respectively, and used according to the manufacturers’ recommendations.

**Table 1. Description of treatments used in the broiler study**

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (oxidized oil and PUFA source)¹</th>
<th>Control (nonoxidized oil and no PUFA source)²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HO</td>
<td>VE</td>
</tr>
<tr>
<td>Oxidized soybean oil, %</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Nonoxidized soybean oil, %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PUFA source, %</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin E, mg/kg</td>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>Antioxidant blend, mg/kg</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

¹HO: high oxidant diet with vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% oxidized oil, 3% polyunsaturated fatty acid (PUFA) source; VE: HO supplemented with vitamin E level at 200 IU/kg; AOX: HO supplemented with an antioxidant blend (AB, ethoxyquin and propyl gallate) at 135 mg/kg; VE+AOX: HO supplemented with vitamin E level at 200 IU/kg and AB at 135 mg/kg.
²SC: standard control, vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% nonoxidized soy oil, no PUFA source supplement; PC: positive control, SC supplemented with AB at 135 mg/kg.
³Oxidized oil was heated to 95°C to reach peroxide value of 180 mEq/kg of oil, provided by Novus International Inc., St. Charles, MO.
⁴Trevera, algae-based product, provided approximately 55.57% crude fat (containing 41% docosahexaenoic acid), 4,000 kcal of ME/kg, and 16.7% protein, Novus International Inc., St. Charles, MO.
⁵Agrado Plus, an antioxidant blend of ethoxyquin and propyl gallate, Novus International Inc.
Muscle Vitamin A and E

Vitamin A and vitamin E concentrations in the breast muscle were determined by HPLC following the procedures described by Arnaud et al. (1991) and Katsanidis and Addis (1999). Briefly, a 0.25-g muscle sample was added with standard tocopherol, retinol, and ascorbic acid, followed by saponification solution [55% 2H5OH in distilled water and 11% KOH (wt/vol)]. The muscle sample was heated at 80°C for 15 min for saponification. After extraction with cyclohexane containing 0.01% of butylated hydroxytoluene (BHT), samples were evaporated and the residue was reconstituted in ethanol with 0.01% BHT. Samples then were transferred into an Eppendorf tube and centrifuged for 5 min at 13,000 × g at 4°C and then analyzed by chromatography (Agilent Technologies HPLC system 1100 series, Santa Clara, CA) using an Agilent 18 Hypersil 125 mm × 4.0 mm column. A 25-µL injection volume was used. Flow rate was 1.5 mL/min with mobile phase methanol:water of 95:5. The UV detection was at 325 nm for vitamin A and 294 nm for vitamin E.

RNA Extraction, Reverse Transcription, and Real-Time PCR

A 20 to 30 mg aliquot of each liver sample was weighed, placed into a 2-mL microcentrifuge tube along with 5-mm stainless-steel beads and 600 µL of lysis buffer, and homogenized using the TissueLyser II system.
Carcass Characteristics and Meat Quality

Objective color measurements were determined on the left side breast muscle surface, using a portable Minolta CR300 colorimeter (Ramsey, NJ) with D65 illuminant, 0° observer and calibrated against a standard white tile (Holmer et al., 2009). Mean L* (lightness), a* (redness), and b* (yellowness) values were collected from 3 separate locations on the surface of the breast. The left sides of the breast muscle samples were used for drip loss analysis. Breast fillets were individually weighed, sealed in plastic bags, stored at 4°C for 24 h, and then reweighed (Saenmahayak et al., 2010). The right side breast muscle samples were frozen, ground, and homogenized. For pH determination, samples were added in 5 mM iodoacetate at 10 mL/g (Solomon, 1987). Muscle lactate concentration was determined using an enzyme analytical method (Hammelman et al., 2003). First, a 100-mg sample was added in 1 mL of 0.5 M perchloric acid, centrifuged (13,000 × g) for 5 min at 4°C, and 600 µL of supernatant was transferred to 150 mL of KOH (2 M). After a 1:1 dilution, 50 µL of the extract was added to 1.5 mL of 0.1 M reaction buffer (200 mM Tris- hydrazine, 0.4 mM NAD, pH 9.0). The OD1 (OD value before lactate dehydrogenase was added) value was measured by absorbance at 340 nm in triplicate, and OD2 (OD value after the reaction with lactate dehydrogenase) value was obtained after adding lactate dehydrogenase (40 µL; 4 IU/sample; Sigma Chemical, Perth, WA) and incubated for 2 h at 25°C. Absorbance values associated with muscle lactate were determined by subtracting OD1 from OD2 and quantified by lactate standards. Lactate concentration was reported as micromoles per gram of muscle.

Statistical Analysis

Data were analyzed using the GLIMMIX procedure of the Statistical Analysis System (9.2 SAS Institute Inc., Cary, NC). Pen was the experimental unit (n = 10). Growth performance and plasma data were conducted via repeated measures by day. The model included the fixed effects of dietary treatment and sampling day. Means and SE calculations were determined using the least squares means statement, with the slice option to separate treatment means by sampling day. Other data were analyzed using GLM procedure. Tukey’s multiple comparison test was conducted at a significance level of α = 0.05.

RESULTS

Growth Performance

Growth performance results within individual diet phases and cumulative phases of the experiment are summarized in Table 3. When performance was assessed on d 10, d 21, and the first 21 d cumulatively, compared with the SC chickens, a combination of oxidized oil plus PUFA source improved growth with greater BW and least ADG and ADFI in HO, VE, AOX, and VE+AOX treatments. However, this trend did not continue in the finisher phase (d 22 to 42). Chickens in AOX and VE+AOX showed the heaviest BW from d 10 on. Both
treatments had greater ADG than chickens in SC and PC treatments from d 0 to 10 and from d 10 to 21. The AOX and VE+AOX treatments had the best G:F from d 0 to 10 and AOX alone had better G:F compared with the HO, VE, SC, and PC treatments from d 22 to 42 and throughout the 42-d experimental period (P < 0.001). The VE treatment birds fell behind other treatments in BW, ADG, and ADFI in the finisher phase (P < 0.001).

**Plasma AGP, Uric Acid, and TBARS**

The high oxidant treatments had higher AGP levels compared with the control diets fed birds (SC and PC) on d 10 and VE+AOX treatment had higher AGP levels compared with the controls on d 21 (Figure 1A); however, there was no difference among treatments on d 42. There was a greater concentration of uric acid in AOX, SC, and PC treatments compared with HO and VE+AOX treatments on d 10 (P = 0.01, Figure 1B). However, the difference among treatments did not continue during the later phases, as measured on d 21 and 42 (P > 0.05).

On all sampling days, the levels of TBARS in the AOX and VE+AOX treated birds were comparable with those of the SC and PC treated birds (Figure 1C). The TBARS levels in the HO and VE treatments were greater than AOX, SC, and PC treatments on d 21, and VE treatment alone was greatest on d 42.

**Breast Muscle Vitamins A and E**

The concentrations of vitamin A and E in the breast muscle are summarized in Table 4. It seems that the high oxidant treatments did not have significant effect on the concentrations of vitamins A and E. As expected, on d 21, a high dosage of vitamin E supplemented to the VE birds resulted in the greatest vitamin E concentration in the breast muscle, followed by the VE+AOX treatment (P < 0.001). On d 42, the VE birds still exhibited a greater concentration of vitamin E, as did the PC birds. The vitamin E concentration in the muscle of the VE+AOX birds was comparable with that of the HO, AOX, and SC groups. The concentration of muscle vitamin A was greater in the PC birds compared with the SC birds on d 21, whereas there was no significant difference among treatments on d 42.

**Antioxidant Enzyme Gene Expression in the Liver**

Most of these enzymes did not differ among treatments (data not shown), except that SOD showed greater expression in SC birds compared with the HO birds (2.04 vs. 1.09; P < 0.05). On d 21, the expression of SOD tended (P = 0.093) to be less in HO birds, as well. On d 7, the SC birds tended to have a greater GSH-Px activity (P = 0.094).

### Table 3. Growth performance across treatments

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0–10</td>
<td>HO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 0 BW, g</td>
<td>44.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>18.38B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADFI, g</td>
<td>26.10B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.700B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 10 BW, g</td>
<td>227.93B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>57.05AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADFI, g</td>
<td>87.50B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.652</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 21 BW, g</td>
<td>855.44B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>100.90AB</td>
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<td>ADFI, g</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.562CD</td>
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<td></td>
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<tr>
<td>d 22–42</td>
<td>HO</td>
<td></td>
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<tr>
<td>d 0 BW, g</td>
<td>2,974.35B</td>
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<td>ADG, g</td>
<td>38.63B</td>
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<td>ADFI, g</td>
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<tr>
<td>G:F</td>
<td>0.664</td>
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<td></td>
</tr>
<tr>
<td>d 0–42</td>
<td>HO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG, g</td>
<td>60.77AB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADFI, g</td>
<td>118.82A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G:F</td>
<td>0.587CD</td>
<td></td>
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</tr>
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</table>

A–D Means within rows that do not have a common superscript differ significantly (P < 0.01).

1HO: high oxidant diet with vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% oxidized oil, 3% polyunsaturated fatty acid (PUFA) source; VE: HO supplemented with vitamin E level at 200 IU/kg; AOX: HO supplemented with an antioxidant blend (AB, ethoxyquin and propyl gallate) at 135 mg/kg; VE+AOX: HO supplemented with vitamin E level at 200 IU/kg and AB at 135 mg/kg; SC: standard control, vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% nonoxidized soy oil, no PUFA source supplement; PC: positive control, SC supplemented with AB at 135 mg/kg.
Carcass Characteristics and Meat Quality

The individual carcass yields were not significantly different among the treatments, except for the tender yield. The PC birds had larger tender yield compared with the HO and VE+AOX treatments (6.92 vs. 5.47 and 5.48, $P = 0.007$, data not shown).

There were no significant treatment differences in $a^*$ and $L^*$ in the breast muscle, but the VE treatment had a lower $b^*$ compared with the VE+AOX, SC, and PC birds (3.27 vs. 6.34, 5.93, and 5.55, $P = 0.003$, data not shown). The ultimate pH of the HO fed birds in the breast muscle was lower than the VE+AOX birds, and the HO-fed birds showed a greater drip loss compared with the VE and AOX birds ($P < 0.01$, Figure 2). Muscle samples from the VE+AOX fed birds had greater pH, which was consistent with lower observed lactate level compared with the HO and PC birds. The drip loss of the individual VE and AOX treatments were less than the HO treatment (1.33, 1.24, vs. 2.63%, $P = 0.007$); however, the combination of the two did not have an additive effect.

**DISCUSSION**

Polyunsaturated fats and oils in animal diets are prone to oxidation. A large body of research suggests that the addition of oxidized oil negatively affects weight gain and feed conversion ratio in broilers (Cabel et al., 1988; Lin et al., 1989; Engberg et al., 1996; Wang et al., 1997; Ajum et al., 2004; McGill et al., 2011; Tavarez et al., 2011). In the present study, better growth performance occurred in the first 4 treatments supplemented with a PUFA source at 3% (pure DHA approximately 0.68%) in the presence of oxidized oil. This may be due to greater digestibility of unsaturated fat (Crespo and Esteve-Garcia, 2001; Newman et al., 2002; Ferrini et al., 2008). Also, it is likely that DHA had beneficial effects on growth during early growth phases. As reported, fish oil as a PUFA source added at 2 and 4% (containing DHA 0.22 and 0.44% in the diet) increased broiler BW gain from d 1 to 38 by 5.6 and 4.2%, respectively, and final BW by 6.5 and 4.6%, respectively (López-Ferrer et al., 2001). However, when DHA levels in the diet reached 0.72% by adding 8.2% fish oil, there was no significant growth improvement in broilers (López-Ferrer et al., 1999). In the current study, the DHA level was less than 0.72%, which explains why high oxidant treatments showed beneficial effects on growth. This effect did not continue in the finisher phase, which is probably due to compensatory growth of SC birds during the later production phase. In general, the addition of AB in the diet stimulated growth independent of the use of oxidized oil and PUFA source. This is in line with previous findings where its main compound ethoxyquin improved growth performance (Cabel et al., 1988; Dibner et al., 1996; Wang et al., 1997) and the supplementation of AB had growth performance benefits in boilers (Tavarez et al., 2011).

![Figure 1](https://academic.oup.com/ps/article-abstract/93/7/1649/1540182)
When added at 200 mg/kg level in the presence of oxidized oil and DHA, vitamin E possibly acted as a prooxidant as reflected by poorer growth in the finisher phase. In this current model, an excessive supplementation of dietary vitamin E could not effectively improve growth performance, which may have resulted from an imbalance of antioxidants, such as vitamins C and E. Due to a relatively low vitamin C level in the diet, vitamin E may not have been regenerated, and led to the accumulation of vitamin E radicals (radicals derived from α-tocopherol), which can act as prooxidants (Hamre et al., 1997). Similarly, Englmaierova et al. (2011) reported a decreased BW by d 35 of feeding with a greater dietary vitamin E level at 100 mg/kg under the nonstressed condition.

Oxidative stress induces lipid peroxidation and peroxidation products are formed after radical attack at the broken double bonds (Fellenberg and Speisky, 2006). The concentration of TBARS in blood is used as a biomarker of radical-induced damage and endogenous lipid peroxidation. In this study, restoration of plasma TBARS to normal levels with the AOX treatment may be due to an enhancement of the body’s antioxidant defense system. A greater TBARS level in the VE birds on d 42 probably suggests a prooxidant effect of vitamin E when fed at high dietary concentration in the current model.

A functioning antioxidant system is required, especially during conditions of oxidative stress. Uric acid is a primary nitrogen waste product in most animals, but it works as one of the nonenzymatic antioxidants in humans and birds (Lin et al., 2008). It functions as a protective component against oxidative stress and reduces tissue damage associated with ROS production (Carro et al., 2010). Greater uric acid in the AOX, SC, and PC treatments of this study indicates an enhanced nonenzymatic system compared with the HO and VE+AOX treatments in the early growth phase (d 10).

Table 4. Vitamins E and A concentrations in the breast muscle

<table>
<thead>
<tr>
<th>Item</th>
<th>HO</th>
<th>VE</th>
<th>AOX</th>
<th>VE+AOX</th>
<th>SC</th>
<th>PC</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
<tr>
<td>d 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E, mg/kg</td>
<td>1.07C</td>
<td>11.27A</td>
<td>1.19C</td>
<td>6.01B</td>
<td>1.51C</td>
<td>1.52C</td>
<td>0.34</td>
<td>&lt;0.001</td>
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<tr>
<td>Vitamin A, µg/kg</td>
<td>62.00AB</td>
<td>56.40AB</td>
<td>46.75AB</td>
<td>43.20AB</td>
<td>28.00B</td>
<td>143.60</td>
<td>20.34</td>
<td>0.404</td>
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<tr>
<td>d 42</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Vitamin E, mg/kg</td>
<td>1.06B</td>
<td>11.39A</td>
<td>1.53B</td>
<td>2.01B</td>
<td>1.14B</td>
<td>9.76A</td>
<td>0.54</td>
<td>&lt;0.001</td>
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<tr>
<td>Vitamin A, µg/kg</td>
<td>118.80</td>
<td>93.80</td>
<td>117.00</td>
<td>154.40</td>
<td>143.80</td>
<td>143.60</td>
<td>20.34</td>
<td>0.404</td>
</tr>
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</table>

A–C Means in rows with no common superscript differ significantly (P < 0.01).

1HO: high oxidant diet with vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% oxidized oil, 3% polyunsaturated fatty acid (PUFA) source; VE: HO supplemented with vitamin E level at 200 IU/kg; AOX: HO supplemented with an antioxidant blend (AB, ethoxyquin and propyl gallate) at 135 mg/kg; VE+AOX: HO supplemented with vitamin E level at 200 IU/kg and AB at 135 mg/kg; SC: standard control, vitamin E level at NRC requirement of 10 IU/kg (NRC, 1994), 3% nonoxidized soy oil, no PUFA source supplement; PC: positive control, SC supplemented with AB at 135 mg/kg.

Under stress, an acute phase protein response can restore homeostasis and restrict microbial growth in an antibody-independent manner (Murata et al., 2004). A common acute phase protein in avian species (Lee et al., 2010), AGP, exerts its inflammatory and immunomodulating properties under stress (Hochepied et al., 2003). In this study, a greater concentration of AGP in the high oxidant diet treatments of this study reflects a greater stress status compared with the control groups (SC and PC) on d 10.

Oxidative stress induces lipid peroxidation and peroxidation products are formed after radical attack at the broken double bonds (Fellenberg and Speisky, 2006). The concentration of TBARS in blood is used as a biomarker of radical-induced damage and endogenous lipid peroxidation. In this study, restoration of plasma TBARS to normal levels with the AOX treatment may be due to an enhancement of the body’s antioxidant defense system. A greater TBARS level in the VE birds on d 42 probably suggests a prooxidant effect of vitamin E when fed at high dietary concentration in the current model.

A functioning antioxidant system is required, especially during conditions of oxidative stress. Uric acid is a primary nitrogen waste product in most animals, but it works as one of the nonenzymatic antioxidants in humans and birds (Lin et al., 2008). It functions as a protective component against oxidative stress and reduces tissue damage associated with ROS production (Carro et al., 2010). Greater uric acid in the AOX, SC, and PC treatments of this study indicates an enhanced nonenzymatic system compared with the HO and VE+AOX treatments in the early growth phase (d 10).

The concentration of vitamin E in the breast muscle in VE-treated birds was expected to be greater than other treatments. Antioxidant inclusion in the diet has been shown to reduce cellular consumption of vitamin E (Bartov and Bornstein, 1972, 1981; Tavarez et al., 2011). The greater vitamin E concentration in the PC-treated birds suggests a sparing effect of AB for vitamin E in nonoxidized oil treatment. However, in the high oxidant diet treatments (HO, VE, AOX, and VE+AOX), the sparing effect was not significant. Pre-
vious work has also suggested a sparing effect of ethoxyquin for vitamin A (Bartov and Bornstein, 1972). However, in this study, the vitamin A sparing effect of AB was only found in the PC treatment when it was compared with the SC treatment on d 21.

Most of the antioxidant enzymes in the liver did not differ on d 7, 14, and 21, which again suggests that the oxidative status in the liver was moderately altered. The lower expression of SOD in the HO treatment indicates suppressed antioxidant capability in those birds.

Cellular and subcellular membranes high in PUFA are susceptible to oxidation. When membrane integrity is disturbed by free radicals, cellular water may leak out, causing greater drip loss (Mahan, 2001). Compared with HO treatment, a relatively lower drip loss of VE and AOX treatments suggests they have the potential to protect muscle cell membrane integrity from damage induced by high oxidants. During the conversion of muscle to meat, lactic acid builds up in the tissue, leading to a reduction in pH. The lower pH in the VE+AOX treatment was consistent with a greater concentration of lactate compared with the HO and PC treatments in this study. Once the pH has reached the isoelectric point of the major proteins, especially myosin (approximately 5.4), they lose their ability to attract and bind water. In addition, the electrostatic forces that help maintain myofilament space are also reduced near the isoelectric point. Therefore, interfila-

In summary, compared with the SC diet, feeding broilers diets containing oxidized oil and a PUFA source had some beneficial effects on early phase growth. Dietary addition of AOX alone moderately restored the whole body antioxidant capability as measured by uric acid and TBARS. The addition of AB alone and high VE reduced drip loss, but VE may exert a prooxidant property in the finisher phase as measured by growth.

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Alpha(1)-acid glycoprotein: An acute phase protein with inflammatory 


