Supplementing antioxidants to pigs fed diets high in oxidants: I. Effects on growth performance, liver function, and oxidative status

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ABSTRACT: The objective of the study was to determine the effects of a dietary antioxidant blend (ethoxyquin and propyl gallate) and vitamin E on growth performance, liver function, and oxidative status in pigs fed diets high in oxidants. Crossbred barrows (n = 100, 10.91 ± 0.65 kg BW, 36 ± 2 d of age, Landrace × Duroc) were allotted to 5 treatments on the basis of BW (5 replicate pens per treatment, 4 pigs per pen). Treatments included 1) HO, high-oxidant diet containing 5% oxidized soybean oil and 10% PUFA source (providing 2.05% docosahexaenoic acid in the diet), 2) VE, the HO diet with 11 IU/kg of added vitamin E, 3) AOX, the HO diet with antioxidant blend (135 mg/kg), 4) VE+AOX, the HO diet with both vitamin E and antioxidant blend, and 5) SC, a standard corn-soy control diet. The trial lasted for 118 d; on d 83, the HO diet pigs were switched to the SC diet because the animals were displaying very poor health. Compared with SC pigs, HO pigs had decreased ADG (0.92 vs. 0.51 kg for d 26 to 55, 1.29 vs. 0.34 kg for d 56 to 82; P < 0.05) and ADFI (1.84 vs. 0.96 kg for d 26 to 55, 3.41 vs. 1.14 kg for d 56 to 82; P < 0.05). However, switching the HO pigs to the SC diet resulted in HO pigs having a greater ADG than VE-fed pigs from d 83 to 118 (0.90 vs. 0.60 kg; P < 0.05). The antioxidant blend restored pig performance to a level similar that of pigs fed the SC diet (P > 0.05) with greater G:F for the entire period (0.44 vs. 0.38; P < 0.05). A greater liver to BW ratio was found in HO compared with other treatments on d 55 and in VE on d 118. Total bilirubin concentration in plasma of HO pigs on d 55 was greater than that in VE+AOX pigs (P < 0.05), whereas on d 118, bilirubin concentration in VE was higher than those in VE+AOX and SC (P < 0.05). A similar trend was observed in aspartate transaminase. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) and carbonyl were elevated (P < 0.05) in the HO pigs compared with the SC pigs on d 55 but not on d 118. Liver TBARS and carbonyl concentrations showed a similar trend, except that HO pigs had the greatest carbonyl concentration on d 118. Pigs fed AOX diets had plasma and liver TBARS and carbonyl concentrations similar to those fed SC diets. In the oxidative stress model used in this study, dietary addition of antioxidant blend or antioxidant blend + vitamin E was effective in improving growth, liver function, and plasma markers of oxidative stress, but VE alone was not.

Key words: antioxidant, carbonyl, growth performance, liver function, pig, TBARS

INTRODUCTION

Lipids are a concentrated energy source, supporting fast and efficient growth in food animals, but they are prone to oxidation. In particular, diets with a high concentration of PUFA have increased potential for nutrient oxidation (Chahboun et al., 1990). The elevated cost and greater use of feed grains for biofuel production have increased the use of by-products with greater concentrations of PUFA, enhancing the potential for nutrient oxidation and oxidative stress in meat animals.
Oxidative stress is defined as the imbalance of pro-oxidants and antioxidants. The excessive free radicals react with membrane lipids and proteins to induce cellular and tissue damage (Machlin et al., 1959; Valko et al., 2006). Exogenous antioxidants may be needed to protect animals from oxidative stress. Synthetic antioxidants, such as ethoxyquin and propyl gallate, effectively reduced rancidity in feed (Njobeh et al., 2006). Ethoxyquin is a free radical scavenger antioxidant that is commonly used to preserve vitamins and lipids in various foods and feed (Thorisson et al., 1992; de Koning, 2002). Ethoxyquin improved growth performance and markers of oxidative status in pigs (Dibner et al., 1996; Fernandez-Duenas, 2009; Harrell et al., 2010). Propyl gallate is a polyphenol widely used to chelate iron ions, which catalyze the formation of oxidative radicals (Madhavi and Salunkhe, 1995). We hypothesized that a commercial combination of these 2 antioxidants (antioxidant blend, ethoxyquin and propyl gallate) could effectively protect pigs from oxidative stress induced by dietary oxidants. Therefore, the objective of this study was to evaluate the effects of an antioxidant blend on performance, liver function, and oxidative status of pigs fed diets high in oxidants.

MATERIALS AND METHODS

The complete protocol was approved by the Virginia Tech Institutional Animal Care and Use Committee (IACUC).

Animals and Experimental Design

One hundred castrated male weanling pigs (21 ± 2 d of age, Landrace × Duroc) from a commercial source (Murphy-Brown LLC, Waverly, VA) were placed in an environmentally controlled nursery room at the Virginia Tech Tidewater Agricultural Research and Extension Center swine unit in Suffolk, VA. Pigs were fed a pretest diet for 15 d before the start of the experiment. After the pretrial period, all pigs were individually tagged, weighed, and randomly assigned to 5 dietary treatments from blocks designed to balance initial weight across treatments. Each treatment was assigned to 5 pens with 4 pigs per pen. Dietary treatments were as follows: 1) HO, high-oxidant diet containing 5% oxidized soybean oil (peroxide value of approximately 180 mEq/kg of oil) and 10% of a commercial PUFA source (TREVERA, NOVUS International Inc., St. Charles, MO), 2) VE, the HO diet with 11 IU/kg of added vitamin E (total diet vitamin E of 24 IU/kg), 3) AOX, the HO diet with the antioxidant blend (ethoxyquin and propyl gallate at 135 mg/kg), 4) VE+AOX, the HO diet with both vitamin E and antioxidant blend, and 5) SC, a standard corn-soy control diet. The HO, VE, AOX, and VE+AOX diets were formulated to create the potential to impose dietary oxidative stress. Additional details on the oxidized soybean oil and PUFA source for these diets are presented in Table 1. The SC diet was formulated to approximate a corn-soybean-based diet typical of North American production conditions. Analyzed fatty acid profiles of the experimental diets and commercial PUFA source are presented in a companion paper (Lu et al., 2014). All experimental diets were fed on an ad libitum basis throughout the experiment.

The experimental diets (Table 1) were prepared by making a basal diet and then adding vitamin E and/or antioxidant blend. The SC diet with nonoxidized soybean oil was made separately. The supplemental vitamin E level for the SC, VE, and VE+AOX diets was 11 IU/kg, according to NRC (1998) recommendations. The antioxidant blend (AGRAO PLUS) was provided by NOVUS International Inc. To prepare the oxidized oil, soybean oil was heated to 95°C and was oxidized by continuously bubbling air into the oil at a rate of 80 L/min for up to 72 h. Peroxide values were determined hourly according to AOCS (2007) methods (peroxide value acetic acid chloroform method, Cd853) so that they reached approximately 180 mEq/kg of oil.

The whole experiment included 5 diet formulation phases: Starter I (d 1 to 10), Starter II (d 11 to 25), Grower I (d 26 to 55), Grower II (d 56 to 82), and Finisher (d 83 to 118). Pigs were individually weighed, and pen feed consumption was determined during the final day of each diet formulation phase, after which the subsequent diet formulation feed was added to the feeders. Because of very poor health and performance, the HO diet pigs were switched to the SC diet at the beginning of the Finisher phase on d 83 as a recovery measure in fulfillment of IACUC requirements. Growth was assessed and plasma was collected at defined time points throughout the 118-d study. After the Starter II phase, pigs were relocated from nursery pens (0.91 × 1.22 m) to finishing pens (1.22 × 2.44 m).

Sample Preparation

At the beginning of the study, 2 pigs from each pen were randomly selected for blood sampling. Blood samples were collected from the jugular vein into 10-mL EDTA vacuum tubes on d 25, 55, and 118. One pig from each pen was randomly selected for tissue collection on d 25, and another pig was randomly selected for tissue collection on d 55; the pigs supplying blood samples were sacrificed and sampled on d 118. There were 4 pigs per pen from d 1 to d 25, 3 pigs per pen from d 26 to 55, and 2 pigs per pen from d 56 to 118. The left central lobe of the liver from each sacrificed pig was flash frozen using liquid nitrogen and was then stored with plasma at −80°C until assays were conducted. On d 118, the remaining 50 pigs were individually identified by tattoo and transported 430 km to the Virginia Tech Meat Science Center processing facility for harvest, sampling, and data collection. The pigs were in transit for 7.5 h.
Thiobarbituric Acid Reactive Substances and Carbonyl

Thiobarbituric acid reactive substances (TBARS) were determined using a commercial assay kit (Cayman Chemical Company, Ann Arbor, MI). For this assay, malonaldehyde (MDA) reacted with thiobarbituric acid (TBA), forming the MDA-TBA product in acidic conditions and at high temperatures (90°C to 100°C), and was measured colorimetrically at 540 nm. Sample MDA concentration was compared with a MDA standard curve. The concentration of TBARS was expressed as plasma MDA concentration (in μM) and tissue homogenate (in μmol/g protein) according to a standard curve. Carbonyl was determined using a protein carbonyl kit (Cayman Chemical Company). With this method, 2,4-dinitrophenylhydrazine (DNPH) reacted...
with protein carbonyl, forming a Schiff base to produce the corresponding hydrazone, which was detected colorimetrically at 372 nm. Protein concentration was determined using a Pierce BCA protein assay kit (Pierce Biotechnology, Rockford, IL). Protein concentration (expressed as mg/mL) was compared with a known BSA concentration standard curve measured colorimetrically at 562 nm.

**Liver Biochemical Parameters**

Plasma γ-glutamyl transpeptidase (GGT) and aspartate transaminase (AST) assay kits were purchased from Teco Diagnositics (Anaheim, CA). The activity of GGT was determined using a kinetic kit at 405 nm. The GGT enzyme catalyzes the transfer of a γ-glutamyl group from γ-glutamyl-p-nitroanilide. Measuring the rate of liberation of p-nitroaniline is directly related to GGT activity. The liver function enzyme AST catalyzes the conversion of oxoglutarate to oxaloacetate, which reacts with a diazonium salt and thus produces a color complex that can be measured at 530 nm. Total bilirubin (TB) and alanine transaminase (ALT) concentrations in the plasma were detected using kits from Bioassay Systems (Hayward, CA). The concentration of TB was measured through a red product from the reaction of bilirubin with diazotized sulfanilic acid at 530 nm. The ALT activity assay was based on the quantification of a pyruvate product by ALT. In this assay, pyruvate and NAD were converted to lactate and NADH by the enzyme lactate dehydrogenase, so the decrease in NADH absorbance at 340 nm was proportional to ALT activity.

**Statistical Analysis**

Data were analyzed using the Glimmix procedure of SAS (version 9.2; SAS Inst. Inc., Cary, NC). Pen was the experimental unit (n = 5 per treatment). For growth performance and plasma indicators, analyses were conducted using repeated measures by sampling day. The model included the fixed effects of dietary treatment, sampling day, and their interaction. Means and SE calculations were determined using the least squares means statement, with the slice option to separate treatment means by sampling day. Tukey’s multiple comparison test was conducted at a significance level of α = 0.05. Pearson correlation coefficients were used to determine the relationships between liver to body weight ratio and growth rate and between TBARS and carbonyl concentrations.

**RESULTS**

On d 71, a pig receiving the VE diet treatment died. All other pigs remained on the study through d 118 of the growth trial.

**Performance**

Growth performance results within individual diet phases of the experiment are summarized in Table 2. Interactions between dietary treatment and sampling day for BW, ADG, ADFI, and G:F were significant (P < 0.05). During the Starter I and Starter II phases, there were no significant differences among treatments for BW, ADG, ADFI, and G:F. Within the Grower I phase, pigs fed the HO diet grew at a slower rate (P < 0.05) than the other treatments. This pattern continued to the end of the Grower II phase on d 82. However, as a result of switching the HO treatment pigs to the SC diet, during the Finisher phase (d 83 to 118) the HO pigs recovered and showed a greater ADG than the VE pigs. The reduced growth rate for HO was associated with pronounced reduction in daily feed consumption (P < 0.05) compared with that on other treatments during the grower phases. At the end of Grower II, HO-fed pigs showed the poorest G:F. These observations provide confirmation that the experimental model to create oxidative stress was effective.

The growth rate of the VE pigs was comparable to AOX pigs and the VE+AOX pigs during the Grower I phase. However, at the end of Finisher phase, the growth rate of VE pigs was the poorest among treatments. The ADFI for VE pigs was less than that for the SC pigs during the Grower I, Grower II, and Finisher phases. The growth rates of AOX and VE+AOX pigs were comparable to those of SC pigs during the Grower I, Grower II, and Finisher phases. The G:F of the AOX-fed pigs during the Grower I phase and the VE+AOX-fed pigs during the Finisher phase were better than that for the SC pigs.

The cumulative growth performance data during the first 25 d of the experimental period were similar among treatments (data not shown). However, during d 1 through 82, the HO pigs had a reduced growth rate compared with pigs in other treatment groups. After d 82 the HO pigs were fed the SC diet as a rescue procedure. Under this condition the HO pigs recovered such that for the entire 118-d period cumulative growth rate was similar for the HO- and VE-fed pigs, but pigs on both of these treatments had poorer overall growth rate than the AOX-, VE+AOX-, and SC-fed pigs. Similarly, pigs on the HO and VE treatments did not consume as much feed as the pigs on the SC treatment during the d 1 to 82 and d 1 to 118 periods. The feed efficiency of AOX pigs was better than that of SC pigs from d 1 to 82. For the whole trial period, the AOX and VE+AOX treatment pigs had greater feed efficiency than SC and HO treatment pigs. However, it must be acknowledged that the AOX and AOX+VE diets had greater energy density than the SC diet. There appeared to be no additive effects of vitamin E and AOX during any cumulative period.
Liver to BW Ratio and Biochemical Parameters

A greater liver to BW ratio was found in HO compared with other treatments on d 55 (Fig. 1). At harvest after d 118, the liver to BW ratio of VE-fed pigs was greater than that of AOX, VE+AOX, and SC pigs. The ratio was negatively correlated with growth rate ($r = -0.81; P < 0.001$; data not shown), suggesting liver stress or dysfunction in pigs experiencing severe oxidative stress.

Plasma GGT, TB, ALT, and AST concentrations were measured on d 25, 55, and 118 (Table 3). Interactions between dietary treatment and sampling day for GGT, TB, and AST were significant ($P < 0.05$). Total bilirubin concentration in the plasma of HO pigs on d 55 was greater
TBARS and Carbonyl Concentrations in the Plasma and Liver

The TBARS and carbonyl concentrations in the plasma are summarized in Table 4. Interactions between dietary treatment and sampling day for plasma TBARS and carbonyl concentrations were significant (P < 0.05). The lipid peroxidation product TBARS was significantly elevated in the plasma of HO pigs on d 55. After the HO pigs were switched to the SC diet on d 83, the TBARS concentration determined for HO pigs on d 118 was restored to a concentration similar to that in the SC pigs. Plasma from pigs fed the VE diet had the greatest TBARS concentration on d 118. A similar pattern was observed in plasma carbonyl concentration, which is an indicator of protein oxidation. Carbonyl concentration was correlated with TBARS concentration (r = 0.72; P < 0.001; data not shown).

Table 3. Plasma concentrations of γ-glutamyl transpeptidase (GGT), total bilirubin (TB), alanine transaminase (ALT), and aspartate transaminase (AST) across treatments

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>d25</td>
<td>HO^2 VE AOX VE+AOX SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT, IU/L</td>
<td>13.79 16.97 13.65 10.66 15.05</td>
<td>2.83 0.631</td>
<td></td>
</tr>
<tr>
<td>TB, mg/dL</td>
<td>2.95 2.62 2.94 2.29 2.62</td>
<td>0.42 0.873</td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>35.57 31.44 37.95 34.00 31.38</td>
<td>4.69 0.873</td>
<td></td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>18.12 19.88 23.75 16.33 25.27</td>
<td>3.29 0.366</td>
<td></td>
</tr>
<tr>
<td>d55</td>
<td>HO^3 VE AOX VE+AOX SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT, IU/L</td>
<td>34.73 14.98 31.18 26.51 29.59</td>
<td>7.67 0.562</td>
<td></td>
</tr>
<tr>
<td>TB, mg/dL</td>
<td>2.92 1.43 1.53 0.90 1.08</td>
<td>0.37 0.035</td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>22.13 32.41 30.71 33.11 28.31</td>
<td>4.21 0.552</td>
<td></td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>59.81 26.21 20.83 22.39 19.55</td>
<td>4.11 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>d118</td>
<td>HO^3 VE AOX VE+AOX SC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT, IU/L</td>
<td>11.35 17.60 18.56 18.28 10.18</td>
<td>3.79 0.492</td>
<td></td>
</tr>
<tr>
<td>TB, mg/dL</td>
<td>0.30 1.67 0.84 0.2 0.3</td>
<td>0.28 0.002</td>
<td></td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>23.25 30.52 21.65 24.11 30.26</td>
<td>2.27 0.051</td>
<td></td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>52.66 36.34 21.32 21.35 21.55</td>
<td>2.34 &lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

a,bMeans in the same row with no common superscript differ (P < 0.05).
^1HO = high-oxidant diet containing 5% oxidized soybean oil (peroxide value of approximately 180 mEq/kg of oil and a dietary peroxide value of 9 mEq/kg) and 10% of a commercial PUFA source containing 55% crude fat, of which 36% is docosahexaenoic acid, VE = the HO diet with 11 IU/kg of added vitamin E, AOX = the HO diet with an antioxidant blend (ethoxyquin and propyl gallate, 135 mg/kg), VE+AOX = the HO diet with both vitamin E and antioxidant blend, SC = a standard corn-soy control diet with nonoxidized oil and no PUFA source. The HO treatment pigs were switched to the SC diet after d 82 as an intervention for very poor health and performance in that treatment group. There were 5 pens in each treatment (n = 5). One pig from each pen was sacrificed to provide samples for d 55, and 2 pigs from each pen were scarified to provide data for d 118. a,bMeans for the same sampling day with no common superscript differ (P < 0.05).

^2The HO treatment pigs were switched to the SC diet after d 82 as an intervention for very poor health and performance in that treatment group.

^3ND: not detectable.

The TBARS and carbonyl concentrations in the liver are summarized in Fig. 2 and 3. The HO pigs had the greatest concentration on d 55 (P = 0.001), whereas VE pigs tended to have the greatest concentration on d 118 (P = 0.10). The protein oxidation in the liver was relatively less in all pigs except HO pigs on d 55, which is similar to the result in the plasma. However, HO pigs maintained the greatest carbonyl concentration through d 118, whereas AOX-added treatments showed a carbonyl concentration similar to that of the SC treatment. Carbonyl concentration was correlated with TBARS concentration (r = 0.58; P < 0.001; data not shown).

DISCUSSION

The reduction in ADG, ADFI, G:F, and BW in HO pigs became apparent by the Grower I phase. In the current model, oxidized soybean oil used at 5% of the diet is 1 source of dietary oxidants. In this study we did not measure the influence of PUFA addition on diet oxidation directly, but high supplementation of PUFA, which is very susceptible to oxidation (Chahboun et al., 1990),
Table 4. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) and carbonyl levels across treatments

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>HO²</th>
<th>VE</th>
<th>AOX</th>
<th>VE+AOX</th>
<th>SC</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS, µM/mL</td>
<td>d 25</td>
<td>2.60</td>
<td>3.14</td>
<td>2.11</td>
<td>2.28</td>
<td>2.30</td>
<td>0.59</td>
<td>0.771</td>
</tr>
<tr>
<td></td>
<td>d 55</td>
<td>14.76</td>
<td>a</td>
<td>11.36</td>
<td>b</td>
<td>9.50</td>
<td>b</td>
<td>8.25</td>
</tr>
<tr>
<td></td>
<td>d 118</td>
<td>2.76</td>
<td>b</td>
<td>10.56</td>
<td>b</td>
<td>4.21</td>
<td>b</td>
<td>4.10</td>
</tr>
<tr>
<td>Carbonyl, nmol/mL</td>
<td>d 25</td>
<td>25.99</td>
<td></td>
<td>20.39</td>
<td></td>
<td>22.32</td>
<td></td>
<td>20.74</td>
</tr>
<tr>
<td></td>
<td>d 55</td>
<td>9.74</td>
<td>a</td>
<td>58.66</td>
<td>b</td>
<td>26.23</td>
<td>c</td>
<td>21.49</td>
</tr>
<tr>
<td></td>
<td>d 118</td>
<td>25.94</td>
<td>b</td>
<td>71.53</td>
<td>a</td>
<td>24.21</td>
<td>b</td>
<td>21.31</td>
</tr>
</tbody>
</table>

¹ Means in the same row with no common superscript differ (P < 0.05).
² HO = high-oxidant diet containing 5% oxidized soybean oil (peroxide value of approximately 180 mEq/kg of oil and a dietary peroxide value of 9 mEq/kg) and 10% of a commercial PUFA source containing 55% crude fat, 36% of which is docosahexaenoic acid, VE = the HO diet with 11 IU/kg of added vitamin E, AOX = the HO diet with an antioxidant blend (ethoxyquin and propyl gallate, 135 mg/kg), VE+AOX = the HO diet with both vitamin E and antioxidant blend, and SC = a standard corn-soy control diet with nonoxidized oil and no PUFA source. There were 5 pens in each treatment (n = 5), with 4 pigs per pen from d 1 to 25, 3 pigs per pen from d 26 to 55, and 2 pigs per pen from d 56 to 118. Two designated pigs provided the plasma samples.

Figure 2. Thiobarbituric acid reactive substances (TBARS) level in the liver on d 55 and 118. HO = high-oxidant diet containing 5% oxidized soybean oil (peroxide value of approximately 180 mEq/kg of oil and 9 mEq/kg in the diet) and 10% of a commercial PUFA source (providing 55% crude fat, of which 36% is docosahexaenoic acid), VE = the HO diet with 11 IU/kg of added vitamin E, AOX = the HO diet with an antioxidant blend (ethoxyquin and propyl gallate, 135 mg/kg), VE+AOX = the HO diet with both vitamin E and antioxidant blend, SC = a standard corn-soy control diet, with nonoxidized oil and no PUFA source. There were 5 pigs in each treatment (n = 5), with 4 pigs per pen from d 1 to 25, 3 pigs per pen from d 26 to 55, and 2 pigs per pen from d 56 to 118. Two designated pigs provided the plasma samples. The HO treatment pigs were switched to the SC diet after d 82 as an intervention for very poor health and performance in that treatment group.

Figure 3. Carbonyl level in the liver on d 55 and 118. HO = high-oxidant diet containing 5% oxidized soybean oil (peroxide value of approximately 180 mEq/kg of oil and 9 mEq/kg in the diet) and 10% of a commercial PUFA source (providing 55% crude fat, of which 36% is docosahexaenoic acid), VE = the HO diet with 11 IU/kg of added vitamin E, AOX = the HO diet with an antioxidant blend (ethoxyquin and propyl gallate, 135 mg/kg), VE+AOX = the HO diet with both vitamin E and antioxidant blend, SC = a standard corn-soy control diet, with nonoxidized oil and no PUFA source. There were 5 pigs in each treatment (n = 5), with 4 pigs per pen from d 1 to 25, 3 pigs per pen from d 26 to 55, and 2 pigs per pen from d 56 to 118. Two designated pigs provided the plasma samples. The HO treatment pigs were switched to the SC diet after d 82 as an intervention for very poor health and performance in that treatment group.

Growth suppression from oxidized lipids has been well documented in a large number of studies (Cabel et al., 1988; Lin et al., 1989; Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010; McGill et al., 2011). Feeding pigs an oxidative diet requires supplementation of increased concentrations of antioxidants. In this study, supplementing vitamin E at the NRC (1998) requirement of 11 IU/kg was not effective in preventing oxidative stress in the Grower II and Finisher phases. This result was clearly demonstrated in the performance data of ADG from d 56 to the end of the study. In the final phase, the VE pigs showed a slower growth rate than the HO pigs, apparently the result of the HO pigs being switched to the SC diet after d 82 for welfare reasons. Because of the free radical scavenging has increased dietary content of oxidized lipid and a series of toxic aldehydes (Esterbauer, 1993; Blokhina et al., 2003). Aldehydes, ketones, acids, esters, and polymerized oils are direct products of oxidation and can result in reduced dietary energy values and a rancidity condition that ultimately destroys acceptability and usefulness of fats and oils (Sherwin, 1978). Products of oxidation may also reduce the absorption and utilization of the fat-soluble vitamins and may react with other nutrients in the diet, such as proteins and amino acids, impairing their biological values (Frankel, 1984). Moreover, free radicals, which are induced by increased amounts of oxidized lipids, can attack macromolecules in the body such as lipids, protein, and nucleic acids (Valko et al., 2006) and can thus impair animal health and growth (Miller et al., 1993).
The change in the liver marker enzyme AST. Taken together, these observations allude to the occurrence of liver damage. Bilirubin, the end product of red blood cell turnover, is produced by Kupffer cells lining the sinusoid and is transported to the hepatocyte for conjugation (Yen, 2001). In this study, the elevation in plasma bilirubin concentrations might be due to erythrocyte hemolysis. The erythrocyte membrane is susceptible to oxidative stress because of its high content of polyunsaturated fatty acids. High doses of free radicals or oxidation products from the oxidative stress diet probably caused a destruction of the erythrocyte membrane structures (Nakazawa and Nagatsu, 1980). This result was consistent with the results from Senthilkumar et al. (2006), who reported that oxidative stress alters the concentrations of the nonenzymatic antioxidant glutathione and antioxidant enzymes in erythrocytes, leading to an elevation of bilirubin due to erythrocyte hemolysis.

Lipid is the macromolecule most susceptible to free radical damage, which results in cleavage of the double bonds. Through a chain reaction mechanism, lipid peroxidation products are formed (Follenberg and Speisky, 2006). The concentration of TBARS in blood and tissues can generally be used as a biomarker of radical-induced damage and endogenous lipid peroxidation. Restoration to nearly normal concentrations of MDA by antioxidant blend may be due to an enhancement of the body’s antioxidant defense system. This result is consistent with a previous swine study in which an elevation of TBARS was found after oxidized oil was applied, whereas AOX reduced plasma and muscle TBARS (Boler et al., 2012).

The secondary products of lipid oxidation will induce protein oxidation as well, which may cause fragmentation and conformational changes in the secondary and tertiary structures of proteins and their function. Amino acid residues are major targets of free radicals (Follenberg and Speisky, 2006). Carbonyl derivatives are an important oxidation by-product of such residues, which is indicative of the extent of oxidative damage affecting the amino acid residues. Liver was sensitive to protein oxidation as measured by carbonyl concentration in this study. A greater change in carbonyl concentration was found in the liver of aged rats (Breusing et al., 2009). Rats fed a diet containing heated oil developed different degrees of apparent liver damage regardless of the chemical properties of the ingested oils (Totani et al., 2008). Protein oxidation, or protoperoxidation, is a slower and less extended process than lipid oxidation, which was reflected by the greatest carbonyl concentration being in the liver of HO pigs, although their diet was switched to SC at d 82. Overall, supplementing the high-oxidant diets with antioxidant blend resulted in an oxidative status similar to that of pigs fed the SC diet, as measured by concentrations of TBARS and carboxyls.

Collectively, feeding pigs diets with increased oxidant concentrations had negative effects on growth. The antioxidant capability of ethoxyquin and the iron chelating action of propyl gallate (Thorisson et al., 1992; de Koning, 2002), antioxidant blend improved growth performance regardless of vitamin E supplementation. This result is consistent with an observed improvement in ADG and ADFI due to antioxidant blend supplementation in pigs fed dry diet. The growth performance results were confirmed in an early study in which an elevation of TBARS concentration was found after oxidized oil was applied, whereas AOX reduced plasma and muscle TBARS (Boler et al., 2012).

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Collectively, feeding pigs diets with increased oxidant concentrations had negative effects on growth. The antioxidant blend improved growth performance regardless of vitamin E supplementation. This result is consistent with an observed improvement in ADG and ADFI due to antioxidant blend supplementation in pigs fed dry diet.
dant blend-added treatments significantly restored pig performance to a level similar to that of pigs fed the SC diet with greater feed efficiency than SC. The negative effects of the oxidative stress diet resulted in a greater liver to BW ratio with higher bilirubin concentrations and AST activities in the plasma, which suggests impaired liver function as a result of these treatments. The concentrations of TBARS and carbonyl in the plasma and liver were significantly elevated, suggesting both lipid oxidation and protein oxidation. The addition of antioxidant blend, providing ethoxyquin and propyl gallate, protected pigs fed the high-oxidant diet from oxidative stress, whereas supplementation of vitamin E alone at the NRC requirement of 11 IU/kg diet could not prevent the negative effects of the oxidative stress diet.

**LITERATURE CITED**


