Effect of vitamin E source, natural versus synthetic, and quantity on serum and tissue (alpha)-tocopherol concentrations in finishing swine


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Effect of vitamin E source, natural versus synthetic, and quantity on serum and tissue α-tocopherol concentrations in finishing swine


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ABSTRACT: Relative vitamin E status of pigs fed natural or synthetic vitamin E was evaluated based on serum and tissue α-tocopherol concentrations. Individually fed finishing gilts at a BW of 70.5 kg (n = 24) were allotted to dietary treatments based on initial BW. The 5 dietary treatments consisted of a positive control diet using synthetic vitamin E acetate (Syn E Ac) supplemented at 22 mg/kg, and 4 dietary levels of natural vitamin E acetate (Nat E Ac) supplemented at 6.71, 8.33, 11.00, and 16.18 mg/kg of diet. Before initiation of the 32-d experiment, pigs were fed a non-vitamin E-fortified diet for 30 d. Diets were formulated to contain true ileal digestible lysine of 0.9 and 0.8% for the pretest and test diets. Serum samples were collected on d 15 and 32, whereas tissue samples were collected on d 32 for α-tocopherol analysis. Serum α-tocopherol concentrations on d 15 and 32 were greater (P < 0.05) in pigs fed 8.33, 11.00, or 16.18 mg/kg of Nat E Ac than pigs fed 22 mg/kg of Syn E Ac. When compared with pigs fed 22 mg/kg of Syn E Ac, α-tocopherol concentrations were greater (P < 0.05) in 6 tissues (heart, kidney, spleen, liver, lung, and adipose) in pigs fed 16.18 mg/kg of Nat E Ac; greater (P < 0.05) in heart, kidney, spleen, liver, and adipose tissue in pigs fed 11.00 mg/kg of Nat E Ac; and greater (P < 0.05) in spleen in pigs fed 8.33 mg/kg of Nat E Ac. As dietary Nat E Ac increased from 6.71 to 16.18 mg/kg, serum α-tocopherol increased linearly (P < 0.01) on d 15 and 32 of the experiment. Increasing dietary Nat E Ac linearly increased (P < 0.05) α-tocopherol concentrations for heart, kidney, spleen, liver, and lung. These results indicate that Nat E Ac was an effective vitamin E source and its relative bioavailability was substantially greater than 1.36 for finishing swine when compared with Syn E Ac.

Key words: bioavailability, pig, stereoisomer, tocopherols, vitamin E

INTRODUCTION

Most dietary inclusions of vitamin E are the acetylated synthetic form of α-tocopherol [i.e., all-rac-α-tocopheryl acetate (Syn E Ac), also commonly known as DL-α-tocopheryl acetate]. Another vitamin E source is natural source vitamin E [d-α-tocopherol, d-α-tocopheryl acetate, or RRR-α-tocopheryl acetate (Nat E Ac)], which is derived from vegetable oils. Synthetic vitamin E is composed equally of 8 stereoisomers of α-tocopherol, differing at the 2′, 4′, and 8′ positions on the phytyl tail (R or S configuration), resulting in RRR, RRS, RSR, RSS, SRR, SSR, SRS, and SSS isomers. The biological activities of these 8 stereoisomers range from 25 to 100% (Blatt et al., 2004), whereas the natural source vitamin E contains only the RRR stereoisomer and has a biological activity of 100% in most mammals (Brigelius-Flohe and Traber, 1999). It has been shown that an α-tocopherol transfer protein in hepatic tissue preferentially binds RRR-α-tocopherol and facilitates the transfer to serum lipoproteins (Sato et al., 1991). The preferential transfer of the RRR stereoisomer by the hepatic α-tocopherol transfer protein seems to explain the greater bioavailability for the RRR-α-tocopherol molecule.

Research indicates that the relative bioavailability of Nat E Ac to Syn E Ac is underestimated in young and adult swine when using the commonly accepted ratio of 1.36:1 (Mahan et al., 2000; Lauridsen et al., 2002; Wiburn et al., 2008). The 1.36 value was initially determined in pregnant rats (Harris and Ludwig, 1949), and its efficacy was extended to various production phases of other animal species without verification. The relative bioavailability of Nat E Ac to Syn E Ac has been estimated to be approximately 2:1 in sows (Lauridsen et al., 2002). The objective of this study was to deter-
mine the retention of α-tocopherol in blood and various body tissues of finishing swine fed diets containing various levels of Nat E Ac as compared with a positive control diet containing Syn E Ac.

MATERIALS AND METHODS

Animal care procedures outlined by the guidelines of the Agricultural Animal Care and Use Committee of the ADM Animal Nutrition Research Center were followed for management, housing, and slaughter procedures. The ADM animal care guidelines are based on the Guide for the Care and Use of Agricultural Animals outlined by the Federation of Animal Science Societies (1999).

**Animals and Diets**

A total of 24 gilts (Newsham Choice Genetics, EB × GP354) were used in this experiment (BW = 70.5 ± 1.3 kg). Five dietary treatments were arranged in a randomized complete block design in 4 or 5 replicates. Because of a limited number of metabolism crates, only 4 gilts were assigned to treatment 5. The 5 dietary treatments consisted of a positive control diet (treatment 1) containing Syn E Ac at 22 mg/kg [2 × NRC (1998)], and 4 dietary levels of Nat E Ac supplemented at 6.71, 8.33, 11.00, and 16.18 mg/kg of diet (treatments 2 through 5, respectively). The Nat E Ac (Nova-E) used in the study was manufactured by Archer Daniels Midland Company (Decatur, IL).

Before the 32-d experiment, gilts were fed a non-vitamin E-fortified diet for 30 d to reduce serum and tissue stores of α-tocopherol and to establish a common baseline for the experimental animals. Corn-soybean meal-based diets were formulated to contain 0.9 and 0.8% true ileal digestible Lys for the 30-d pretest (45 to 70 kg of BW), and the test period (70 to 100 kg of BW), respectively. The true ileal digestible Lys concentrations for these BW exceed NRC recommendations, but are common concentrations used in the US swine industry. The pretest and test diets contained 0.30 mg/kg of added Se from Na selenite. Other vitamins and nutrients met or exceeded current NRC (1998) requirements for the 50- to 120-kg pig. Composition of the basal diets is presented in Table 1.

During the first 23 d of the pretest period, pigs were housed in partially slatted concrete floor pens (4.0 ×
Blood and Tissue Sample Collection

Blood samples (5 to 7 mL) were collected by jugular venipuncture from pigs on d 0, 15, and 32 of the study. Pigs were fasted for 24 h before bleeding. On d 0, 10 pigs were randomly selected for bleeding, whereas all 24 pigs were bled on d 15 and 32. Blood was placed on ice and subsequently centrifuged (3,000 × g for 15 min at 4°C). Serum was collected and stored at −10°C until analyzed for α-tocopherol. At the end of the experimental period, pigs were electronically stunned and killed by exsanguination at a local abattoir. Samples of liver, heart, lung, kidney, spleen, brain, loin, and adipose from the subcutaneous backfat area at the last rib were immediately collected, stored on ice for transport to the laboratory, frozen, and later analyzed for α-tocopherol.

Analytical Methods

Serum and tissue samples were analyzed for their total α-tocopherol content by HPLC based on the procedures of Zaspel and Csallany (1983). Approximately 4 g of tissue was cut from a nonexposed edge of the collected tissue and then prepared for analysis as outlined by Wilburn et al. (2008).

Statistical Analysis

Data were analyzed by ANOVA procedures (Steel and Torrie, 1980) using the GLM procedure (SAS Inst. Inc., Cary, NC), and the model included block and treatment. Treatment least squares means were compared by nonorthogonal contrasts (Syn E Ac treatment to each of the Nat E Ac treatments). Contrast statements with coefficients computed by the IML procedure of SAS were also used to evaluate the linear, quadratic, and cubic response only for the Nat E Ac treatments. Pearson correlation coefficients were determined with the CORR procedure of SAS for d 15 and 32 serum α-tocopherol concentrations vs. d 32 tissue α-tocopherol concentrations. The individual pig was considered the experimental unit for all analyses.

RESULTS

Feed Analysis

The analyzed concentrations of α-tocopheryl acetate were 15.5 mg/kg for the pretest diet. The calculated and analyzed concentrations of α-tocopheryl acetate were 38.1 vs. 38.8, 22.8 vs. 21.5, 24.5 vs. 23.2, 27.1 vs. 26.8, and 32.3 vs. 29.2 mg/kg for treatments 1 through 5 (complete diets), respectively. The calculated and analyzed values were similar, although the exact concentrations of indigenous vitamin E in the basal feeds were unknown. Analyzed concentrations of CP, crude fiber, fat, Ca, and P in experimental diets were similar to calculated values (Table 1).

Serum α-Tocopherol

Average serum α-tocopherol concentration was 1.02 ± 0.09 µg/mL (n = 10) on d 0 of the study. Serum α-tocopherol concentrations were numerically greater in those pigs fed the least Nat E Ac diet than those fed the Syn E Ac diet on d 15 and 32 (Table 2). Serum α-tocopherol concentrations were greater (P < 0.05) in those pigs fed the other 3 Nat E Ac diets when compared with pigs fed the Syn E Ac diet. Serum α-tocopherol concentrations increased linearly (P < 0.01) on d 15 and d 32 as dietary Nat E Ac supplementation increased from 6.71 to 16.18 mg/kg.

Tissue α-Tocopherol

As supplemental Nat E Ac level increased from 6.71 to 16.18 mg/kg, α-tocopherol concentrations in the heart (P < 0.01; Table 2), kidney (P < 0.01), spleen (P < 0.01), liver (P < 0.01), and lung (P < 0.03) increased linearly. When compared with pigs fed 22.00 mg/kg of supplemental Syn E Ac, the α-tocopherol concentrations in all tissues analyzed were generally similar (Table 2) in pigs fed the 6.71 mg/kg of supplemental Nat E Ac, but were greater (P < 0.05) in spleen, loin, and adipose tissues in pigs fed supplemental Nat E Ac at 8.33 mg/kg, greater (P < 0.05) in heart, kidney, spleen, liver, and adipose tissues in pigs fed supplemental Nat E Ac at 11.00 mg/kg, and greater (P < 0.05) in heart, kidney, spleen, liver, lung, and adipose tissues in pigs fed supplemental Nat E Ac at 16.18 mg/kg. Each of the 8 tissue α-tocopherol concentrations were positively correlated (P < 0.04) with serum α-tocopherol concentrations on d 15 and 32 (Table 3).

Performance

Pigs fed 22.00 mg/kg of Syn E Ac performed similarly to those fed the 4 diets containing different levels
of Nat E Ac (Table 4). Increasing dietary addition of Nat E Ac from 6.71 to 16.18 mg/kg had no effects (P > 0.05) on overall ADG, ADFI, G:F, or final BW during the 32-d experimental period.

**DISCUSSION**

Pigs were fed a non-vitamin E-fortified diet 1 mo before starting the study to reduce body stores of α-tocopherol and to increase their sensitivity to the dietary vitamin E supplementation. The test model appeared to be effective because serum α-tocopherol concentrations were less on d 0 than on d 15 and 32, and serum α-tocopherol concentrations differed significantly among dietary treatments on d 15 and 32.

Vitamin E requirements for swine are expressed as international units per kilogram in swine NRC (1998). This requires conversion of vitamin E from weight (mg) to an international unit basis. The recognized conversion factor is 1 mg = 1 IU for Syn E Ac, whereas 1 mg = 1.36 IU for Nat E Ac (NRC 1998). The 4 Nat E Ac treatments were designed to have equally spaced ratio changes of 0.64 (3.28, 2.64, 2.00, and 1.36). These ratios led us to supplement Nat E Ac at 6.71, 8.33, 11.00, and 16.18 mg/kg of diet for treatments 2 through 5, respectively, when Syn E Ac was supplemented at 22 mg/kg for treatment 1. Using the commonly accepted 1.36 conversion factor, vitamin E supplementation would be 9.13, 11.33, 14.96, and 22.00 IU/kg when diets were supplemented with the 4 levels of Nat E Ac for treatments 2 to 5, respectively. Supplementation of 9.13 IU/kg was below the recommended vitamin E requirement of 11 IU/kg for pigs with BW of 10 to 120 kg (NRC 1998).

Linear increases in tissue α-tocopherol concentrations were observed as supplemental Nat E Ac increased from 6.71 to 16.18 mg/kg in heart, kidney, spleen, liver, and lung, indicating Nat E Ac was an effective source of vitamin E in swine diets. The linear improvement was observed when dietary Nat E Ac supplementation increased from 0.83 to 2 times the NRC (1998) requirement of 11 IU/kg. Chung et al. (1992) reported that supplementing Nat E alcohol at 3 and 6 times NRC requirements linearly increased α-tocopherol concentrations for serum, heart, liver, lung, and loin in nursery pigs. Mahan et al. (2000) observed elevated concentrations of serum and tissue α-tocopherol when dietary

### Table 2. Simple correlation of α-tocopherol concentrations between d 32 tissues and serum at d 15 and 32

<table>
<thead>
<tr>
<th>Item</th>
<th>d 15 serum</th>
<th>d 32 serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.78</td>
<td>0.82</td>
</tr>
<tr>
<td>Heart</td>
<td>0.82</td>
<td>0.82</td>
</tr>
<tr>
<td>Lung</td>
<td>0.47</td>
<td>0.47</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.83</td>
<td>0.84</td>
</tr>
<tr>
<td>Brain</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>Loin</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td>Adipose</td>
<td>0.61</td>
<td>0.51</td>
</tr>
</tbody>
</table>
Table 4. Effect of dietary vitamin E source and level on growth performance of finishing swine

<table>
<thead>
<tr>
<th>Item</th>
<th>Vitamin E source and level, mg/kg</th>
<th>P-value (Nat-E)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment No.</td>
<td>Syn-E²</td>
<td>Nat-E³</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.00</td>
<td>6.71</td>
<td>8.33</td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>16.18</td>
<td></td>
</tr>
<tr>
<td>BW, kg</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>0.81</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>ADFI, kg</td>
<td>2.11</td>
<td>2.06</td>
<td>2.11</td>
</tr>
<tr>
<td>G:F, kg/kg</td>
<td>0.38</td>
<td>0.41</td>
<td>0.40</td>
</tr>
</tbody>
</table>

1 The number of animals was 5, 5, 5, 5, and 4 for treatment 1 to 5, respectively.
2 Syn-E = all-rac-α-tocopheryl acetate (synthetic vitamin E, DSM Nutritional Products, Parsippany, N.J.).
3 Nat-E = RRR-α-tocopheryl acetate (natural vitamin E, Archer Daniels Midland, Decatur, IL).
4 Lin = linear; Quad = quadratic.
5 BW at the end of 32-d experiment. Average initial BW was 70.5 kg.
6 Feed intake of gilts was restricted to that of the gilt with smallest voluntary intake within each BW block at the start of the study. The daily offered feed amount was increased by 0.05 kg/pig every 4 d.

Natural vitamin E for swine

Nat E Ac addition was increased from 30 to 60 IU/kg in reproducing sows.

Tissue α-tocopherol concentrations at d 32 were positively correlated with serum α-tocopherol concentrations during the middle (d 15) and at the end (d 32) of the study, implying that these tissues actively incorporate α-tocopherol into cellular components in direct proportion to the serum concentrations. Although correlation between tissue and serum α-tocopherol concentrations at the same collection time has been reported (Jensen et al. 1990), it is believed that this study is the first to report that α-tocopherol concentrations of tissues collected at d 32 were correlated with those of serum collected at d 15. The positive correlation between tissue and serum α-tocopherol concentrations indicates that serum α-tocopherol concentrations might be used to indicate dietary treatment effects on tissue α-tocopherol concentrations when studying vitamin E nutrition of swine.

Alpha-tocopherol concentrations of serum and the 6 tissues (heart, kidney, spleen, liver, lung, and adipose) were consistently greater for pigs fed the diet containing 16.18 mg/kg of Nat E Ac than those for pigs fed the diet containing 22 mg/kg of Syn E Ac, indicating that relative bioavailability of Nat E Ac to Syn E Ac was greater than 1.36. If the 1.36 traditional bioavailability conversion for Nat E Ac to Syn E Ac was correct, tissue concentrations in pigs fed the Syn E Ac would be equal to tissue concentrations in pigs fed the 16.18 mg/kg of Nat E Ac. Recent studies have reported relative bioavailability of Nat E Ac vs. Syn E Ac to be greater than 1.36 in nursery pigs (Ching et al., 2002), growing-finish pigs (Anderson et al., 1995), sows (Mahan et al., 2000; Lauridsen et al., 2002), and in dairy cows (Meglia et al., 2006; Weiss et al., 2009), beef cows (Hidiroglou et al., 1988), and in humans (Burton et al., 1998). Collectively, the evidence indicates that the relative bioavailability of Nat E Ac is greater than 1.36 times that of Syn E Ac. Furthermore, relative bioavailability of Nat E Ac to Syn E Ac appears to be at least 2.00 because α-tocopherol concentrations for 5 of 8 tissues and serum at 2 collection times were greater for pigs fed supplemental Nat E Ac at 11.00 mg/kg than for pigs fed Syn E Ac at 22.00 mg/kg. However, current experimental design and methodology do not provide a specific estimation of relative bioavailability. Relative bioavailability of at least 2:1 between Nat E and Syn E has been reported in swine, beef cows, and humans. Chung et al. (1992) reported a relative bioavailability ratio of 2.44 between the alcohol form of Nat E and acetate form of Syn E in nursery pigs. Also, Lauridsen et al. (2002), using deuterium-labeled vitamin E in sows, estimated that the bioequivalence ratio of Nat E Ac over Syn E Ac was similar to 2:1. The Nat E Ac bioavailability was estimated to be over 2 times greater than that of Syn E Ac in beef cows (Hidiroglou et al., 1988) and approximately twice as potent in humans (Burton et al., 1998).

The natural vitamin E from plant sources contains only the RRR-α-tocopherol. The synthetic all-rac-α-tocopherol consists of 8 stereoisomers (RRR, RRS, RSS, RSR, SRR, SSR, SRS, and SSS, equimolar at 12.5% for each stereoisomer). These isomers have a common chromanol ring structure but differ in the direction of rotation (right or left, R or S) at the 2′, 4′, and 8′ carbons in the phytal tail of the tocopherol molecule, which result in significantly different 3-dimensional molecular structures. The 3-dimensional configuration of the α-tocopherol molecule seems to determine whether it can be transferred by hepatic α-tocopherol transfer protein (Esterbauer et al., 1991).

All 8 α-tocopherol isomers appear to be absorbed in the intestine (incorporated into chylomicrons) where they are transported to the liver by the lymphatic system (Traber et al., 1990). In the liver, RRR-α-tocopherol is preferentially incorporated into very low density lipoproteins by hepatic α-tocopherol transfer protein (Sato et al., 1991; Arai et al., 1993; Kaempf-Rotzoll et al., 2003). It is by this mechanism that the α-tocopherol isomers with the R configuration in the 2′
carbon position (RRR, RRS, RSS, and RSR) are preferentially retained in body tissues, whereas most other α-tocopherol isomers having the S configuration in the 2′ carbon position (SRR, SSR, SRS, and SSS), and the β-, γ-, and δ-tocopherols are excreted via the bile into the intestinal tract for elimination in feces (Traber and Kayden, 1989; Kayden and Traber, 1993). Thus, the α-tocopherol transport protein preferentially binds RRR-α-tocopherol to a greater extent than the other 2′R isomers (Traber and Araí, 1999), resulting in apparent differences in bioavailability among the 4 2′R isomers (Weiser and Vecchi, 1982; Weiser et al., 1996).

No evidence has been reported in the literature to suggest that 2′S isomers can be converted to 2′R isomers in vivo. These biological mechanisms help explain why Nat E Ac was more bioavailable than Syn E Ac in this study.

These estimations of relative bioavailability in animals are consistent with the Food and Nutrition Board, Institute of Medicine (2000) that recognizes only the 2′R-stereoisomers of α-tocopherol as contributing to the human vitamin E requirement, whereas other forms of vitamin E (2′S α-tocopherols, β, γ, and δ-tocopherols and tocotrienols) do not contribute toward the human vitamin E requirement because they are poorly recognized by the α-tocopherol transfer protein (α-TPP) in the liver. Similar data for poor utilization of the 2′S α-tocopherol isomers have been reported in dairy cattle (Weiss et al., 2009).

Our experiment demonstrates that various tissues responded differently to the increased supplementations of dietary Nat E Ac. Dietary levels of Nat E Ac had linear effects on α-tocopherol concentrations in heart, kidney, spleen, liver, and lung, but had no significant effects on α-tocopherol concentrations in brain, adipose tissue, and loin. Ingold et al. (1987) reported that the RRR isomer was concentrated in rat brain and exceeded 5 times that of its 2′S (SRR) isomer, indicating that brain α-tocopherol concentrations should have responded to dietary Nat E Ac addition. Jensen et al. (1990) indicated that muscle and fat tissue α-tocopherol concentrations reflected the long-term vitamin E status of the pig and that serum and liver α-tocopherol concentrations reflected the short-term vitamin E status of the pig. Therefore, it is possible that our 32-d study was not sufficiently long enough to have stabilized the vitamin E status in brain, loin, and adipose tissue.

In conclusion, increasing dietary Nat E Ac linearly increased α-tocopherol concentrations for serum, liver, lung, heart, kidney, and spleen. Alpha-tocopherol concentrations of serum and most tissues were consistently greater in pigs fed the 16.18 mg/kg of Nat E Ac diet and several tissues (heart, kidney, spleen, liver, and adipose) had greater α-tocopherol concentrations in pigs fed 11 mg/kg of Nat E Ac diet than in pigs fed 22 mg/kg of Syn E Ac diet, indicating Nat E Ac was an effective vitamin E source. Our results indicate that the relative bioavailability of Nat E Ac was greater than the currently accepted value of 1.36 for finishing swine when compared with Syn E Ac and that the ratio may need to be modified for swine.

LITERATURE CITED


Mahan, D. C., Y. Y. Kim, and R. L. Stuart. 2000. Effects of vitamin E sources (RRR or all-rac-α-tocopheryl acetate) and levels on sow reproductive performance, serum, tissue and milk
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