Effect of the type of dietary triacylglycerol fatty acids on α-tocopherol concentration in plasma and tissues of growing pigs1

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ABSTRACT: A study was performed in growing pigs to evaluate the efficacy of α-tocopherol (Tol) concentration in plasma, muscle, liver, and adipose tissue following dietary supplementation with vitamin E (VE) and various sources of fat. The trial involved 96 piglets weaned at an average of 28 d of age. Piglets were fed for 2 wk a semi-purified diet not supplemented with VE. Piglets were then randomly assigned to 5 isoenergetic semipurified diets with 100 IU/kg VE as dl-α-tocopheryl acetate: a control (CTRL) diet (with no added fat) and 4 other diets containing either 3% linseed oil (LIN), 3% hydrogenated coconut oil (COC), 3% olive oil (OLI), or 3% safflower oil (SAF) representing diets rich in n-3 PUFA, SFA, MUFA, and n-6 PUFA, respectively. After 49 d of treatment, pigs were killed and blood, muscle (longissimus dorsi), adipose tissue, and whole liver (without gallbladder) were collected and analyzed for their Tol concentrations. For all tissues, LIN and SAF diets led to lower (P < 0.02) Tol concentrations as compared to the CTRL diet: –63 and –67%, respectively. α-Tocopherol concentrations in plasma, liver, and adipose tissue were greater (P < 0.001) in the COC group as compared to the CTRL group. The OLI diet led to greater (P < 0.01) liver Tol concentration (+92%) as compared to the CTRL diet but had no significant effect on plasma, muscle, and adipose tissue Tol concentrations. There were significant correlations (P < 0.001) between plasma, muscle, and liver Tol concentrations (r > 0.78). These results show that supplementation with PUFA markedly decreases Tol concentration in blood and tissues of growing pigs, whereas SFA increase Tol content in blood, liver, and adipose tissue. Monounsaturated fatty acids only increase liver Tol concentrations. Therefore, increasing the amount of fat in the diet (from <0.1 to approximately 3.5%) and the type of dietary fatty acids supplemented with VE are key factors with regards to VE concentration in plasma and tissue. The Tol:PUFA needs to be carefully considered to meet the VE pigs requirement and to ensure an optimal Tol meat enrichment.

Key words: bioavailability, dietary fatty acids, muscle, oil, swine, vitamin E

INTRODUCTION

Vitamin E (VE) is an essential nutrient for normal growth, health, and reproduction in most mammals including pigs. Vitamin E is primarily involved in cells to protect lipid structures against peroxidation (Buettner, 1993) and in gene expression as tocopherols downregu-
supplemented into pig diets with a practical upper limit of 6%. Information on the effect of the type of dietary fatty acids on VE concentration in pig is not consistent; some studies have suggested that SFA increase plasma, muscle, and fat Tol whereas PUFA decrease Tol concentration in these tissues (Babinszky et al., 1991; Monahan et al., 1992; Lauridsen, 2010). Conversely, studies did not observe any effect (Hidiroglu et al., 1993; Babinszky et al., 1991). Also, no clear conclusion can be drawn on the effect of dietary MUFA or on specific effects of n-3 vs. n-6 PUFA (Rey et al., 2001; López-Bote et al., 2003). Therefore, the aim of our study was to answer if the type of dietary triacylglycerol (TAG) fatty acids affects blood and tissue Tol concentration in growing pigs.

MATERIALS AND METHODS

The animals were reared and slaughtered in compliance with regulations for use of animals in research, as operated at the Center of Expertise and Research in Nutrition of Adisseo, Commentry, France.

Animals and Diets

A total of 96 male piglets (Large White × Landrace × Pietrain) weaned at 28 ± 1 d of age were used in this experiment. Piglets were obtained from an independent farm in the area of Commentry (France). After arrival at the experimental facilities of Adisseo, animals were fed for 14 d a commercial wheat–soybean meal based diet supplemented with 5 g/kg doxycycline (Cali Pig Doxy 2012; Tellus, St Germain de Salles, France). During the 14 following days, pigs received a semipurified diet not supplemented with fat whereas the 4 other experimental diets were chosen to allow significant variations in plasma and tissue concentrations of VE as linear responses to VE supplementation below 200 IU/kg were reported (Trefan et al., 2011), and compiled absorption studies reviewed by Wang and Leibholz (1990) indicate that the absorption of VE in pigs is not influenced by the dietary source and amount of fat when the diet contains more than 10% fat. In the present study, we decided to apply the inclusion level of 3% fat as it is globally used by the pig industry (FEFAC, 2011). Finished diets (sampled 3 times at d 1, 25, and 49 during the experimental period) and feed-grade oils used as fat sources were analyzed for fat (finished diet only), VE, and phytosterols content, and fatty acids profile was also determined. Live weight and feed intake were recorded at 56 d of age for treatment allocation, after 3 wk of treatment, and the day before slaughtering. During the duration of the trial, health status of pigs was followed. During the growth phase, 3 pigs died: 1 in the CTRL group and 2 in the COC group.

Sample Collection

At the end of the 49-d experimental period, all pigs from each treatment were stunned by electric shock and killed by exsanguination after an overnight fasting period of 14 h. Blood was collected in heparinized tubes (S-Monovette 9 mL LH, reference 02.1065.001; Sarstedt AG and Co., Nümbrecht, Germany). Plasma was obtained by centrifugation at 1,000 × g for 10 min at 4°C and frozen at −20°C for further analyses. Whole liver (without gall bladder) and samples of muscle (longissimus dorsi) and subcutaneous fat samples from a region in the middorsal area (thereafter called “adipose tissue”) were collected, weighed, frozen at −20°C, and analyzed for Tol concentrations.

Laboratory Analysis

Vitamin E Determination. Vitamin E, as Tol, was determined in muscle, liver, and adipose tissue samples after saponification. Biological samples (2 to 5 g for adipose tissue samples, 10 to 15 g for liver, and 5 to 10 g for muscle) were homogenized by adding 10 mL of sodium ascorbate at 200 g/L, 150 mL of ethanol at 95%, and 50 mL of potassium hydroxide at 500 g/L. The samples were then saponified by refluxing during 30 min under N. If, after saponification and cooling, fat or oil was present on the surface of the mixture, additional ethanol and potassium hydroxide were added and the saponification time was extended. The extraction was done with 3 × 100 mL of petroleum ether.
The combined extracts were washed to neutral with water (3 to 4 times with 100 to 150 mL). Water traces were removed by filtering with sodium sulfate and the extract was then evaporated at 40°C using a rotary evaporator. The samples were dissolved in 25 mL of petroleum ether, diluted in 2,2,4-trimethypentane, and then injected (50 μL) into the HPLC system with fluorometric detection (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 1.3 mL/min using a reverse phase Lichrospher SI-100 column (Merck-Millipore S.A.S., Molsheim, France). The mobile phase...
was a mixture of 2,2,4-trimethylpentane:2-propanol (99:1). Diets and plasma Tol concentrations were analyzed by HPLC as outlined by Zaspel and Csallany (1983) and by Liquid chromatography coupled to tandem mass spectrometry as described in Lauridsen et al. (2001), respectively.

**Fatty Acids Profile.** Lipids were extracted from feed samples with petroleum ether and fatty acid methyl esters of this fraction were prepared with boron trifluoride, as described in the NF EN ISO 5509 procedure (ISO, 2000). The fatty acids profile of the samples was then determined by gas chromatography as described in the NF EN ISO 5508 procedure (ISO, 1995).

**Triglycerides and Cholesterol Analysis.** Plasma concentrations of TAG and cholesterol (CHO) were determined using commercial kits based on colorimetric detection (Triglyceride assay kit, reference 10010303; Interchim, Montluçon, France; and Total Cholesterol assay kit, reference STA-384; Cell Biolabs, Inc., San Diego, CA, USA; respectively).

**Phytosterol Analysis.** Individual and total plant sterols in the experimental diets were determined using gas chromatography as described in the standard procedure ISO 12228 (ISO, 1999).

**Statistical Analysis**

Statistical analysis was performed using the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC). The experimental unit was the individual pig and the fixed effect included in the model was the diet. Means of each group were adjusted for the corresponding total VE intake during the experimental period used as covariate. The data were treated as a dose response assay to compare the regressions \( Y = f(\text{dose}) \) between treatments with an Analysis of Covariance, by adding the treatment effect as a class variable to the regressions. All presented means are least-squares means. Differences between least-squares means were tested by the Tukey’s post hoc test for multiple comparisons. Alpha level used for the determination of significance in all comparisons was 0.05. Correlations between Tol concentrations in plasma, muscle, liver, and adipose tissue were performed using the REG procedure of SAS 9.1.

**RESULTS**

**Dietary Content of α-Tocopherol, Fatty Acids, and Phytosterols**

The total VE content of the various diets fed was measured at 3 different times during the experimental period (at d 1, 25, and 49); mean concentrations are shown in Table 2. The total fat determination of the diets showed concentrations of 3.5, 3.4, 3.8, and 3.3% for the LIN, COC, OLI, and SAF diets, respectively. These contents were in accordance with the calculated level of supplementation (Table 1). For the CTRL diet, as there was no addition of fat to the basal diet, the fat content was below the detection limit of the method, set to be at 0.1%, and therefore, the determination of fatty acids profile for this diet was not possible. For the other oil-enriched diets, the analyzed fatty acid profile reflected the expected profile of the fat sources (Table 2). Indeed, the high content of PUFA (63.0%) in the LIN diet was mainly from n-3 fatty acids (47.5%) represented in total by α-linolenic acid (C18:3n-3), whereas the high content of PUFA (52.8%) in the SAF diet was mainly from n-6 fatty acids (51.5%), represented in total by α-linoleic acid (C18:2n-6). The COC diet had the greatest content of SFA (91.7%; Table 2) due to the high proportion of C12:0 (38.3%), C14:0 (16.1%), and C16:0 (13.6%). The OLI diet had the greatest content of MUFAs (75.0%; Table 2) mainly due to the high proportion of oleic acid (C18:1; 71.2%).

Phytosterols level was 0.02% in the LIN diet and not detectable (<0.01%) in the other diets (Table 2).

**Vitamin E Intake**

The growth performance was not affected by treatments \( (P = 0.189) \). The measurement of feed intake, together with the VE recovery in diet, allowed calculating the total quantity of VE consumed by the animals during the whole duration of the experimental period. Differences in VE intake were detected between groups, with greater \( (P < 0.0001) \) levels for the CTRL \( (1.58 \mu g/mL) \) and COC \( (1.55 \mu g/mL) \) groups and lower levels for the LIN \( (0.90 \mu g/mL) \), OLI \( (0.99 \mu g/mL) \), and SAF \( (0.93 \mu g/mL) \) groups. Because of this difference, the mean Tol concentrations in plasma and tissues were adjusted for VE intake and presented as least-squares means in all the manuscript (see the statistical procedure in the Materials and Methods section).

**Plasma Concentrations of α-Tocopherol and Lipids**

The concentrations of plasma Tol (raw values or adjusted for plasma TAG or CHO) were measured at the end of the experimental period (Fig. 1). When considering raw values, there was a difference \( (P < 0.0001) \) within groups (Fig. 1A). The lower concentrations were found in LIN \( (0.51 \mu g/mL) \) and SAF \( (0.33 \mu g/mL) \) groups. The COC \( (2.15 \mu g/mL) \) group showed a greater \( (+35\% \); \( P < 0.0001) \) concentration than the CTRL group. The OLI group \( (1.93 \mu g/mL) \) exhibited an intermediate concentration between the CTRL \( (1.59 \mu g/mL) \) and the COC groups.

The TAG concentration in plasma was not influenced \( (P = 0.249) \) by the dietary treatments (Table 3) whereas plasma CHO concentrations respond to the type of fat in the diet (Table 3). Indeed, the LIN and SAF diets led to the lowest CHO levels \( (60.3 \text{ and } 54.0 \text{ mg/dL}, \text{ respectively; } \)
Table 2. Fatty acids composition and fat, vitamin E, and phytosterols content of the oil-enriched experimental diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Fat source1</th>
<th>Fatty acid2</th>
<th>LIN</th>
<th>COC</th>
<th>OLI</th>
<th>SAF</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>C6:0</td>
<td>0.1</td>
<td>0.8</td>
<td>0.0</td>
<td>0.2</td>
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<tr>
<td></td>
<td></td>
<td>C8:0</td>
<td>0.1</td>
<td>7.0</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C10:0</td>
<td>0.2</td>
<td>5.5</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C12:0</td>
<td>0.3</td>
<td>38.3</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C14:0</td>
<td>0.9</td>
<td>16.1</td>
<td>0.4</td>
<td>1.0</td>
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<td></td>
<td></td>
<td>C16:0</td>
<td>11.8</td>
<td>13.6</td>
<td>12.6</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C16:1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C18:0</td>
<td>4.0</td>
<td>9.7</td>
<td>3.5</td>
<td>4.7</td>
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<tr>
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<td></td>
<td>C18:1</td>
<td>17.0</td>
<td>2.6</td>
<td>71.2</td>
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<td></td>
<td></td>
<td>C18:2</td>
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<td></td>
<td>C18:3</td>
<td>47.7</td>
<td>0.3</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>2.2</td>
<td>1.4</td>
<td>3.8</td>
<td>4.9</td>
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<tr>
<td></td>
<td></td>
<td>Total SFA3</td>
<td>18.3</td>
<td>91.7</td>
<td>17.4</td>
<td>24.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Total MUFA4</td>
<td>18.5</td>
<td>3.4</td>
<td>75.0</td>
<td>22.5</td>
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<tr>
<td></td>
<td></td>
<td>Total PUFA5</td>
<td>63.0</td>
<td>4.9</td>
<td>7.5</td>
<td>52.8</td>
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<td></td>
<td></td>
<td>U:S ratio6</td>
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<td>0.1</td>
<td>4.4</td>
<td>3.0</td>
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<td></td>
<td></td>
<td>Σn-3</td>
<td>47.5</td>
<td>0.3</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σn-6</td>
<td>15.3</td>
<td>4.6</td>
<td>6.8</td>
<td>51.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Σn-6:Σn-3</td>
<td>0.3</td>
<td>15.3</td>
<td>9.7</td>
<td>85.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat, % (as-fed basis)</td>
<td>3.5</td>
<td>3.4</td>
<td>3.8</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin E,8 mg/kg</td>
<td>53.7</td>
<td>71.3</td>
<td>53.3</td>
<td>55.7</td>
</tr>
</tbody>
</table>

Phytosterols, % (as-fed basis) 0.02 <0.01 <0.01 <0.01

Values are mean concentrations of 3 sampling times performed at d 1, 25, and 49 of the experimental period.

1LIN = basal diet supplemented with 3% linseed oil; COC = basal diet supplemented with 3% coconut hydrogenated oil; OLI = basal diet supplemented with 3% olive oil; SAF = basal diet supplemented with 3% safflower oil.

2Weight percentage of total fat.

3Total weight percentage of SFA.

4Total weight percentage of MUFA.

5Total weight percentage of PUFA.

6U:S = total unsaturated fatty acids (MUFA + PUFA):total SFA.

7Analytical values. In Table 1, values shown are theoretical fat content for the oil-enriched experimental diets (Allix software; A-Systems, Versailles, France).

8Total α-tocopherol, that is, free d-α-tocopherol provided by the oils added to the diets plus α-tocopherol added to the diets as dl-α-tocopherol acetate.

$P = 0.775$) whereas the COC diet led to the greatest CHO concentration (91.5 mg/dL). The adjustment of plasma Tol concentration with plasma TAG or CHO concentrations slightly attenuated the differences between groups (Fig. 1B and 1C, respectively), with the difference between the COC and the CTRL groups becoming nonsignificant. Nevertheless, the LIN and SAF groups still had lower lipid-adjusted plasma Tol concentrations than the CTRL group.

**Tissue Concentrations of α-Tocopherol**

The concentrations of Tol in muscle, liver, and fat are shown in Fig. 2.

In the longissimus dorsi muscle, the lowest concentrations were found in the groups fed diets rich in PUFA, that is, the LIN and SAF diets. The Tol concentrations in muscle in the CTRL (5.0 μg/g), COC (5.4 μg/g), and OLI (4.9 μg/g) groups did not differ ($P > 0.09$) and were approximately 3 times greater ($P < 0.0001$) than the concentrations measured in the LIN and SAF groups.

In the liver, the COC and OLI diets led to greater ($P = 0.011$ and $P < 0.0001$, respectively) concentrations, +32 (9.17 μg/g) and +92% (13.42 μg/g), respectively, as compared to the CTRL diet. As for muscle, the lowest Tol concentrations were found in the LIN and SAF groups, with concentrations lower ($P < 0.001$) than 54 (3.2 μg/g) and 61% (2.7 μg/g), respectively, as compared to the CTRL group.
For adipose tissue, the maximum content of Tol was observed with the COC diet (11.0 μg/g), which was greater ($P < 0.01$) than the 4 other diets. The CTRL, LIN, OLI, and SAF diets did not differ ($P > 0.10$).

**Correlations between α-Tocopherol Concentrations in Plasma and Tissues**

The correlations are presented in Table 4. All possible combinations with Tol concentration in plasma and the other studied tissues were correlated. The greatest correlation coefficients were between plasma and muscle or liver, both having an $r = 0.86$, whereas the correlations between adipose tissue Tol and Tol in the other studied tissues were lower, that is, $r < 0.34$.

**DISCUSSION**

The first main observation of this study is that increasing the amount of fat in the diet (from <0.1% in the CTRL diet to approximately 3.5% in the 4 oil-enriched diets) did not systematically increase plasma and tissue concentrations of VE. Indeed, some oil-enriched diets had no significant effect on plasma or tissue VE levels when compared to the CTRL diet while other oil-enriched diets, that is, the PUFA-rich ones, systematically decreased plasma and tissue VE levels. This result was unexpected because previous studies (Hofmann, 1963; Dimitrov et al., 1991; Anwar et al., 2007) have shown that VE absorption efficiency increases as a function of the amount of dietary fat (Bruno et al., 2006). This suggests that the type of fat is a more important factor than the amount of fat to affect VE bioavailability.

The second main finding is that diets rich in PUFA, that is, the LIN and SAF diets, led to reduced concentrations of VE in plasma and in all the studied tissues as compared to the CTRL diet (not supplemented with fat) and also as compared to diets rich in SFA (the COC diet) and MUFA (the OLI diet). This observation is in agreement with previous studies. Indeed, Malm et al. (1976) reported that a supplementation with stripped corn oil (high in n-6 PUFA) tended to diminish serum VE

### Table 3. Concentrations of triglycerides (mg/dL) and cholesterol (mg/dL) in plasma of pigs after 49 d of treatment

<table>
<thead>
<tr>
<th>Item</th>
<th>CTRL</th>
<th>LIN</th>
<th>COC</th>
<th>OLI</th>
<th>SAF</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of pigs</td>
<td>19</td>
<td>19</td>
<td>17</td>
<td>19</td>
<td>19</td>
<td>0.249</td>
</tr>
<tr>
<td>Plasma triglycerides, mg/dL</td>
<td>32.2 ± 4.7</td>
<td>34.1 ± 3.5</td>
<td>26.2 ± 3.3</td>
<td>28.7 ± 5.0</td>
<td>26.3 ± 2.9</td>
<td>0.249</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td>76.6 ± 4.1$^b$</td>
<td>60.3 ± 2.1$^b$</td>
<td>91.5 ± 4.8$^c$</td>
<td>85.5 ± 3.2$^{bc}$</td>
<td>54.0 ± 3.4$^c$</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^a$–$^c$Within a row, least-squares means ($±$SEM) that do not have a common superscript letter differ ($P < 0.05$). The Analysis of variance followed by Tukey test.

CTRL = control (basal diet with no supplemented fat); LIN = basal diet supplemented with 3% linseed oil; COC = basal diet supplemented with 3% coconut hydrogenated oil; OLI = basal diet supplemented with 3% olive oil; SAF = basal diet supplemented with 3% safflower oil.

Figure 2. Tissue vitamin E concentration (μg α-tocopherol/g of fresh tissue) in pigs after 49 d of treatment. $^a$–$^d$For individual tissue, means ($±$SEM) without a common superscript differ ($P < 0.05$). The analysis of variance followed by Tukey test. CTRL = control (basal diet with no supplemented fat; $n = 19$); LIN = basal diet supplemented with 3% linseed oil ($n = 19$); COC = basal diet supplemented with 3% coconut hydrogenated oil ($n = 17$); OLI = basal diet supplemented with 3% olive oil ($n = 19$); SAF = basal diet supplemented with 3% safflower oil ($n = 19$). α-Tocopherol analyses were performed on homogeneous and representative samples of longissimus dorsi muscle, whole liver (without gallbladder), and subcutaneous fat from a region in the middorsal area (named adipose tissue).
These modifications of the physicochemical properties of -3 fatty acids as compared to a control diet with no added fat. In rats (Weiser and Salkeld, 1977; Tijburg et al., 2012), and they do not increase blood CHO (Forsythe et al., 2010). Therefore, MUFA are quite neutral with the mechanisms hypothesized to be involved in the effect of dietary TAG fatty acids on VE stability in the gut, on VE absorption, and on VE transport in the blood. López-Bote et al. (2003) showed that a greater MUFA:PUFA led to a greater VE deposition in muscle but they cannot conclude whether the observed effect was due to a greater proportion of MUFA or to a lower proportion of PUFA.

In each experimental diet, the content of total phytosterols was also measured, as phytosterols are known to decrease VE absorption by competing for absorption (Katan et al., 2003). The very low level measured could not explain the difference observed between treatments. Finally, another interesting finding of this study is the high and significant correlation ($r = 0.86$) between concentrations as compared to a supplementation with stripped lard (high in SFA), although the difference was not significant. Another study also showed that serum VE was lower with PUFA-rich diets (sunflower and fish oil) than with tallow SFA-rich diet (Lauridsen, 2010). Finally, Kouba et al. (2003) measured a 30% decrease of muscle Tol when feeding pigs with 6% crushed linseed (rich in n-3 fatty acids) as compared to a control diet with no added fat. In rats (Weiser and Salkeld, 1977; Tijburg et al., 1997) and in poultry (Surai and Sparks, 2000; Sijben et al., 2002; Villaverde et al., 2008), Tol concentrations was also reduced when the dietary content of long-chain PUFA increased.

Two mechanisms are suggested to explain the negative effect of dietary PUFA on plasma and tissue VE deposition. The first obvious one is the fact that, in presence of PUFA, a fraction of VE is degraded to protect these highly oxidizable fatty acids from oxidation. This can occur at different “locations” where PUFA and VE are in contact. A stability study performed on 6 h where diets were solubilized in a NaCl 0.9% buffer to mimic the intestinal lumen conditions did not show any effect of fat on VE degradation (internal data). Nevertheless, previous studies support this hypothesis by showing that PUFA supplementation increased muscle oxidative stress (Young et al., 2003) and muscle lipid peroxidation (Mahecha et al., 2010). Therefore, VE mobilization may increase with the greater presence of long-chain PUFA. A second mechanism might be related to VE absorption, as PUFA increase the physical size of mixed micelles and decrease their zeta potential when compared to SFA (Gleize et al., 2012). These modifications of the physicochemical properties of micelles may impair the ability of mixed micelles, carrying VE in the gut (Desmarchelier et al., 2013), to cross the unstirred water layer and to reach the enterocytes, leading in a decrease of the absorption rate of VE. Whatever the mechanism or mechanisms involved, it seems to be similar for n-3 and n-6 PUFA. Indeed, we observed similar effects of the 2 PUFA-rich diets with regard to VE tissue deposition while they had very different $\sum n\text{-}6$ to $\sum n\text{-}3$ ratio.

The third main observation of this study was the positive effect of the COC SFA-rich diet on plasma and liver VE concentrations as compared to the CTRL and PUFA-rich diets. This phenomenon can be explained by a greater intestinal absorption. Indeed, a greater bioaccessibility of another lipophilic micronutrient (lutein) was observed in presence of SFA that lead to a lower size of micelles (Gleize et al., 2012). Moreover, because SFA increased plasma and liver CHO, in agreement with previous studies (Allan et al., 2001; Lauridsen, 2010), it is likely that in our study, SFA have increased the blood volume of VE, that is, the lipoproteins. This can partly explain why the adjustment of plasma VE by plasma CHO abolished the observed difference between the CTRL and the COC diets with regard to plasma VE.

<table>
<thead>
<tr>
<th>Dependent variable (y)</th>
<th>Independent variable (x)</th>
<th>Equation</th>
<th>Slope &amp; P-value</th>
<th>Intercept &amp; P-value</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle α-tocopherol</td>
<td>Plasma α-tocopherol</td>
<td>$y = 1.74x + 1.40$</td>
<td>0.11 &amp; ***</td>
<td>0.17 &amp; ***</td>
<td>0.86</td>
</tr>
<tr>
<td>Liver α-tocopherol</td>
<td>Plasma α-tocopherol</td>
<td>$y = 4.95x + 0.50$</td>
<td>0.32 &amp; ***</td>
<td>0.48 &amp; NS²</td>
<td>0.86</td>
</tr>
<tr>
<td>Fat α-tocopherol</td>
<td>Plasma α-tocopherol</td>
<td>$y = 1.55x + 3.50$</td>
<td>0.51 &amp; **</td>
<td>0.73 &amp; ***</td>
<td>0.34</td>
</tr>
<tr>
<td>Muscle α-tocopherol</td>
<td>Liver α-tocopherol</td>
<td>$y = 0.27x + 1.78$</td>
<td>0.02 &amp; ***</td>
<td>0.20 &amp; ***</td>
<td>0.78</td>
</tr>
<tr>
<td>Fat α-tocopherol</td>
<td>Liver α-tocopherol</td>
<td>$y = 0.21x + 3.99$</td>
<td>0.09 &amp; *</td>
<td>0.73 &amp; ***</td>
<td>0.28</td>
</tr>
<tr>
<td>Fat α-tocopherol</td>
<td>Muscle α-tocopherol</td>
<td>$y = 0.77x + 2.83$</td>
<td>0.27 &amp; **</td>
<td>1.02 &amp; **</td>
<td>0.32</td>
</tr>
</tbody>
</table>

1α-Tocopherol analyses were performed on homogeneous and representative samples of longissimus dorsi muscle, whole liver (without gallbladder), and fat (subcutaneous fat samples from a region in the middorsal area).

2NS = no significant difference ($P > 0.05$)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. 

Table 4. Regression equations between α-tocopherol concentration (μg/mL or g) in plasma, muscle, liver, and fat

Concentrations of α-tocopherol were adjusted to a relative concentration of the same fatty acid (TAG fatty acids on VE bioavailability. Monounsaturated fatty acids have no specific effect on zeta potential as compared to other type of fatty acids (Gleize et al., 2012), and they do not increase blood CHO (Forsythe et al., 1980). Therefore, MUFA are quite neutral with the mechanisms hypothesized to be involved in the effect of dietary TAG fatty acids on VE stability in the gut, on VE absorption, and on VE transport in the blood.
plasma VE concentration and muscle VE concentration. Indeed this shows that measuring plasma VE concentration can be used as a very valuable tool to estimate VE concentration in muscle and could serve as a good parameter to estimate of the overall VE status of the animal.

In the past years, research linked to VE in swine production has mainly focused on the effect of its tissue concentration on meat quality. A number of studies have investigated the effects of dietary VE on pork quality traits (Gray et al., 1996); the stabilizing effect of VE on flavor, color, texture, and nutritive value has since been confirmed by many of them (Monahan et al., 1992; Phillips et al., 2001; Souza et al., 2008; Trefan et al., 2011). The results of the present study imply that the use of diets rich in PUFA, either n-3 or n-6, in total substitution of diets rich in MUFA or SFA, can lead to dramatically diminished muscle VE deposition in growing pigs. The amount of VE in the diet should be related to the amount of PUFA. More precisely, Harris and Embree (1963) determined that it is necessary to have a Tol:PUFA equal to or greater than 0.6 to meet VE requirements in many species including pig. In the present study, the Tol:PUFA (around 2.4 and 3.2 for the LIN and SAF diets, respectively) were not sufficient to allow similar VE deposition in tissues of pigs as the amounts deposited in pigs fed diets with <10% PUFA. These results imply that if PUFA-rich diets are used in replacement of SFA- or MUFA-rich diets, it may be necessary to increase by a factor of about 3 the dietary VE level to have the same concentration of VE in muscle. Finally, it is interesting to note that the modulation of muscle VE concentration by dietary fat was achieved within 49 d, making it possible for short-term diet manipulation to optimize meat quality parameters.

**LITERATURE CITED**


Anwar, K., J. Iqbal, and M. M. Hussain. 2007. Mechanisms involved PUFA-rich diets are used in replacement of SFA- or MU-FA-rich diets, it may be necessary to increase by a factor of


