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## A meta-analysis to assess the effect of the composition of dietary fat on $\alpha$ -tocopherol blood and tissue concentration in pigs

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4 **A meta-analysis to assess the effect of the type of dietary triacylglycerols on  $\alpha$ -tocopherol**  
5 **tissue concentration in pigs<sup>1</sup>**

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25 **ABSTRACT:** A meta-analysis based on the results from thirteen selected publications was  
26 performed to assess the effect of dietary fat supplementation (quantity and fatty acid  
27 composition) on  $\alpha$ -tocopherol (TOL) concentration in four pig tissues (blood, liver, muscle  
28 and adipose tissue). Dietary fat supplementation was defined by the quantity of fat added to  
29 the basal diet, its fatty acids profile and its source(s). After standardization of tissue TOL  
30 concentration (as the dependent variable), statistical analyses were performed using multiple  
31 nonlinear regression, data partitioning and partial least squares (PLS) regression with 7  
32 predictor variables: added vitamin E (VE), added fat, polyunsaturated fatty acids (PUFA; %  
33 fat), monounsaturated fatty acids (MUFA; % fat), saturated fatty acids (SFA; % fat), omega-3  
34 fatty acids (n-3; % fat), and omega-6 fatty acids (n-6; % fat). The statistical analyses first  
35 suggested that VE dose in the diet was the main factor that modulates tissue TOL  
36 concentration. The dose-response relationship followed a logarithmic curve, with a saturation  
37 of tissue TOL concentration at around 40-50 mg VE/kg diet. Moreover, the amount of dietary  
38 fat, at least up to 20%, was not linearly correlated with tissue TOL concentration. Considering  
39 the main fatty acid classes, SFA and MUFA were positively associated with tissue TOL  
40 concentration when added VE was below 18.5 mg/kg for SFA and above 18.5 mg/kg for  
41 MUFA. Finally, this study provides more certainties that the inclusion of n-3 fatty acids in the  
42 diet provokes a decrease in tissue TOL concentration.

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44 **Key words:** bioavailability, dietary fat, meta-analysis, swine, vitamin E.

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## INTRODUCTION

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Vitamin E (**VE**) is essential in pig nutrition for normal growth, health, and reproduction. It acts as a powerful antioxidant to protect membrane lipids against peroxidation (Buettner, 1993). To be absorbed, the esterified and stable form of VE, i.e., dl- $\alpha$ -tocopheryl acetate (**TAC**) as generally supplemented to pig diets, is solubilized in mixed micelles and hydrolyzed into free  $\alpha$ -tocopherol (**TOL**) to be released in enterocytes (Desmarchelier et al., 2013). Prior to be uptaken by targeted tissues, TOL is secreted into chylomicrons and high-density lipoproteins (**HDL**) (Reboul et al., 2009). VE is then recovered in blood and in its main storage tissues (i.e., muscle, liver and adipose tissue). Several dietary factors can modulate blood and tissue TOL concentrations such as selenium (Ullrey, 1981), lipids (Nielsen et al., 1973), retinol (Hoppe et al., 1992), copper and iron (Dove and Edwan, 1991), and other antioxidants (Simesen et al., 1982). Borel et al. (2013) have recently highlighted the key role of dietary fat in VE assimilation because they share common pathways for digestion and absorption. It is assumed that dietary fat is required for VE absorption but the optimal amount that maximizes VE absorption has yet to be determined (Thomson and Dietschy, 1981). Published data on the effect of the type of dietary triglycerides on TOL concentration in different tissues are contradictory. Indeed, some studies have suggested that saturated fatty acids (SFA) increase plasma, muscle, and adipose tissue TOL concentration and polyunsaturated fatty acids (PUFA) reduce it (Babinszky et al., 1992; Monahan et al., 1992; Lauridsen, 2010). Conversely, other studies did not show any significant effect of these fatty acids on tissue TOL concentration (Hidiroglou et al., 1993; Babinsky et al., 1991). Furthermore, no clear conclusion can be drawn on the effect of monounsaturated fatty acids (MUFA) or on the effect of the n-3 to n-6 fatty acids ratio on tissue TOL deposition (Rey et al., 2001; López-Bote et al., 2003). Therefore, this study aimed to use meta-analysis to find

73 potential relationships between the type of dietary triglyceride fatty acids and tissue TOL  
74 concentration in pigs.

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## MATERIALS AND METHODS

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### ***Data Sourcing***

80 A literature search and screening process was initially conducted using the following  
81 electronic databases: PubMed, ISI Web of Science and Scirus as search engines to create a  
82 dataset of published studies using the keywords in searching “*fat source*”, “*fat*  
83 *supplementation*”, “*dietary fat*”, “*fatty acids profile*”, “*triglycerides*”, “*triacylglycerols*”,  
84 “*absorption*”, “*bioavailability*”, “*deposition*”, “*vitamin E*”, “*α-tocopherol*”, “*alpha-*  
85 *tocopherol*”, “*pig*”, “*piglet*”, “*gilt*”, “*swine*”, “*hog*”, “*boar*” and “*sow*”. A double-check  
86 was also directly conducted in major animal science peer-reviewed journals. No date  
87 restrictions were placed on the search engines. The literature review was conducted in  
88 December 2013 independently by two individuals, and the respective outputs were compared  
89 and compiled to establish a common selection. Initially, 831 peer-reviewed papers on the  
90 topic of VE in pigs were identified.

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### ***Selection for Inclusion Criteria***

93 The most relevant papers for this meta-analysis described the direct effect of dietary fat  
94 supplementation on tissue TOL concentration in pigs. Nevertheless, studies dedicated to the  
95 effect of fat supplementation on oxidative status, meat quality or fatty acids profile in tissues,  
96 in which TOL was measured in tissues as a secondary indicator, were also included as long as  
97 they were in line with the following inclusion criteria.

98 To be included in the meta-analysis, studies must have been conducted in pigs (*Sus scrofa*  
99 *domesticus*), whatever the breed, gender or physiological stages of life (suckling or weaning  
100 piglets, growing or finishing pigs, gilt, gestating or lactating sow, boar).

101 Each group (control and experimental) required reporting of the number of replications, least  
102 square means and a measure of dispersion. Studies were required to be randomized trials  
103 (factorial designs or complete block).

104 Since Anderson et al. (1995) reported that tissue deposition of TOL is different among VE  
105 isomers in finishing pigs, the form of VE used in the selected studies must be the same, i.e.,  
106 TAC, which is an equimolar mixture of 8 synthetic  $\alpha$ -tocopherol stereoisomers in the acetate  
107 form (Lauridsen et al., 2002). Furthermore, dl- $\alpha$ -tocopheryl acetate is the industrial form of  
108 VE supplementation and is used as a standard in the studies included in the meta-analysis.  
109 One mg of this chemical compound has a biological conversion factor of 1.0 and corresponds  
110 to 1 International Unit (IU) of VE. The amount of supplementation could thus be expressed  
111 either in IU or mg of TAC per kg of compound feed.

112 The length of supplementation period and the dose of VE supplemented were not considered  
113 as restrictive factors. In industrial conditions, supplemental VE has frequently been increased  
114 to 200 IU/kg (Adisseo, 2002), a level substantially higher than 11 to 44 IU/kg diet currently  
115 suggested by NRC (2012). Furthermore, Trefan et al. (2011) concluded from their meta-  
116 analysis that maximum increase of TOL in muscle (*longissimus*) occurred either at low doses  
117 when supplementation duration was long or at high doses when supplementation duration was  
118 short. The inclusion levels in their literature review (13 studies) ranged up to 700 IU/kg.  
119 Therefore, these observations allowed us to consider in the present study a wide range of  
120 dietary VE doses and different duration of supplementation.

121 Also, we decided to focus our analysis on TOL concentration in muscle, liver, adipose tissue  
122 and plasma (or serum) since they are the main tissues of TOL storage (Ullrey, 1981). Muscle  
123 generally referred to *longissimus dorsi*, *thoracis* or *lumborum*.

124 Fat supplementation was critical in the present study as it is the main explicative variable we  
125 targeted to measure the impact on TOL concentration in pig tissues. The term “fat” mainly

126 refers to the solid state of lipids whereas the term “oils” mainly refers to the liquid state of  
127 lipids. Fat in typical swine diet is present as vegetable oils but also as animal-based by-  
128 products or as oils from marine origin. Triglycerides are the main constituents of dietary fat  
129 (approximately 95%; Jørgensen and Fernández, 2000). The practical upper limit of fat  
130 supplementation in regular diets is 2-3% added fat (FEFAC, 2011) but no added fat level  
131 restrictions were placed in the literature search.

132 After leaving out papers that did not meet the selection criteria, a dataset including thirteen  
133 peer-reviewed papers was built. Table 1 shows these selected references with the main  
134 experimental details.

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### 136 ***Standardization Procedures of Tissues TOL Concentration and Dietary Fatty Acids Profile***

137 The measured parameters, either the dependent variable (tissue TOL concentration) or the  
138 independent variables (dietary TAC and fat), must be uniform across the experiments to allow  
139 comparison, as it is recommended by Vernet and Ortigues-Marty (2005). This was achieved  
140 by applying the standardization procedure described as follows.

141 All the experiments considered used the same method to measure VE concentration in the diet  
142 and tissues: VE was extracted, saponified, separated by HPLC and quantified with reference  
143 to a TOL standard, as referred in Buttriss and Diplock (1984). TOL concentration was usually  
144 expressed by mg per kg diet or  $\mu\text{g}$  per g fresh tissue. For tissues, when it was expressed per  
145 unit triglycerides or cholesterol (or total lipids) the raw TOL concentration was calculated  
146 based on the lipid concentration provided. In the present selection of studies, TOL  
147 concentration was measured either in serum or in plasma (using EDTA or heparin as  
148 anticoagulant). Moreover, Jezequel-Cuer et al. (1994) observed significant difference in  
149 human blood TOL concentration depending of the type of collection tube used. As a  
150 consequence, we performed a control experiment on ten pigs to compare their TOL



151 concentrations in serum and plasma coming from the same blood samples. Results (data not  
152 shown) allowed us to conclude that there was no difference between serum and plasma TOL  
153 concentration ( $P = 0.34$ ), and also between plasma concentration obtained from EDTA- or  
154 heparin-coated tubes ( $P = 0.99$ ). Therefore, the TOL concentrations obtained from plasma and  
155 serum were pooled in the statistical analysis and termed “blood”.

156 In each study and for each tissue, i.e., blood, liver, muscle or adipose tissue, TOL  
157 concentration was also expressed as a percentage of the maximum TOL concentration  
158 obtained within each treatment group. This allowed us to decrease the impact of the  
159 variability of the tissue TOL concentration within groups and to highlight more the effect of  
160 treatment (St-Pierre, 2007).

161 For some of the studies included in the analysis, the fatty acids profile of the diet given to the  
162 supplementation group was not analyzed or provided. We then estimated the fatty acid  
163 composition diets using reference values (NRC, 2012). As this last revised version of NRC  
164 did not show any data for n-3 and n-6 fatty acids, we used the previous one (NRC, 1998). We  
165 followed the same procedure when fat supplementation was a mixture of difference fat  
166 sources, taking into account the relative inclusion rate.

167 Data for all independent and dependent variables in the thirteen studies selected in the meta-  
168 analysis were extracted, standardized and entered into a spreadsheet.

169

## 170 ***Database***

171 A comprehensive database was established, satisfying structural requirements outlined by  
172 Vernet and Ortigues-Marty (2005). The database included a detailed description of each  
173 reference (e.g. year of publication, author names), animals used in the experiments (e.g.  
174 gender, breed, number of replications per treatment), diet (e.g. crude fat), fat supplementation

175 (e.g. source, quantity, fatty acids composition), dietary VE (e.g. theoretical supplementation,  
176 analytical recovery), duration of experimental period and collected samples.

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### 178 *Statistical Analyses*

179 Independent variables in the selected studies were considered as fixed variables. The study  
180 effect was considered random because each study is conceptually a random outcome from a  
181 larger population of studies to which inference is to be made. Since the variance between  
182 studies was larger compared to the variance within studies, the study effect was included into  
183 the meta-analysis models. Distribution of random effects was assumed to be normal. The X  
184 matrix contained the following independent variables: VE dose (IU/kg diet), fat content  
185 (g/100g diet), SFA, MUFA, PUFA, n-3 and n-6 levels (g/100g fat). The degree of  
186 interdependence was assessed by calculating the variance inflation factors (VIF) for each of  
187 these 7 predictor variables (St-Pierre and Glamocic, 2000). The Y matrix was composed of  
188 the vector containing TOL concentration in blood, muscle, liver and adipose tissue. A plot  
189 representing the TOL concentration in the different tissues versus the VE dose revealed a  
190 nonlinear relationship between these two factors (Figure 1). A nonlinear function, i.e., an  
191 exponential model, commonly used to describe a biological response with an asymptotic  
192 behaviour (Lambe et al., 2006), was fitted to the data using the PROC NL MIXED procedure  
193 of SAS 9.3 (2012).

194 In order to assess the potential relationship between each of the seven independent variables  
195 and tissue TOL concentration in pigs, we used three different statistical analyses and results  
196 were subsequently compared.

197

198 *Regression.* Data were analysed by a stepwise regression model performed with JMP® 11.2  
199 (SAS, 2012). All predicting variables were entered in the model and then recursively, each

200 non significant ( $P > 0.1$ ) variable was removed from the model. Then, the remaining variables  
201 were entered in a mixed model with the study effect as a random effect in order to account for  
202 inter-study heterogeneity.

203

204 *Partition analysis.* The data have been analysed with JMP® 11.2 (SAS, 2012). The Partition  
205 algorithm recursively partitions data according to a relationship between the X (independent  
206 variables) and the Y values (TOL concentrations in pooled tissues or in each tissue studied  
207 independently), creating a tree of partitions. It finds a set of cuts or groupings of X values that  
208 best predict a Y value. It does this by exhaustively searching all possible cuts or groupings.  
209 These splits (or partitions) of the data are done recursively forming a tree of decision rules  
210 until the desired fit is reached.

211

212 *Partial Least Squares (PLS) regression.* PLS regression is the multivariate regression  
213 extension of principal component analysis. It allows the identification of the X variables  
214 explaining best the variance in a Y variable and performs well when there is multicollinearity  
215 between the X variables (Brindle et al., 2002). It was performed using SIMCA-P12 software  
216 (Umetrics, Umeå, Sweden). Variables were scaled using unit variance scaling. For each  
217 tissue, a PLS regression model comprising all the variables was built and the variable  
218 importance in the projection (VIP) value, representing the contribution of an X-variable to the  
219 PLS model, was calculated for each variable. New PLS regression models were then built,  
220 successively eliminating the X-variable presenting the lowest VIP value. The model  
221 maximizing the explained ( $R^2$ ) and the predicted ( $Q^2$ ) variance and validated following cross-  
222 validation ANOVA and permutations was selected (described in details in Supplementary  
223 Material S1 and Figure S1).

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## RESULTS

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### *Preliminary Graphical Analyses*

Graphical exploration of data allowed us to identify nonlinear relationships between the TOL concentration and the VE dose for all the studied tissues (Figure 1). The mathematical parameters of the exponential models are presented in Table 2. Quadratic and linear models were also fitted to the data but they exhibited lower R-square values and the value of both Akaike's and Bayesian Information Criteria (AIC and BIC, respectively), measuring the relative quality of the models, were higher compared to that of the exponential model (data not shown). The asymptotes (or plateau) showed a saturation of TOL in the tissues. The saturation value was the greatest for liver (16 µg/g). Blood and muscle had the lowest values (2 µg/ml and 5 µg/g, respectively), and adipose tissue had intermediate value (9 µg/g). The growth rate (absolute value in µg vitamin E in tissue per mg vitamin E in diet), that indicates the sensitivity in response to VE dose in feed, was the greatest for blood (0.09) compared to adipose tissue, muscle and liver (0.05, 0.04 and 0.01, respectively).

### *Regression*

Among the seven predictor variables entered in the stepwise regression, the four variables that significantly contribute to R-square were retained in the final model of prediction (after transformation of all data by the exponential model): added VE ( $1.711 \pm 0.075$ ;  $P < 0.001$ ), MUFA ( $0.012 \pm 0.002$ ;  $P < 0.001$ ), SFA ( $0.006 \pm 0.002$ ;  $P < 0.01$ ) and n-6 PUFA ( $0.006 \pm 0.0001$ ;  $P < 0.01$ ). Note that they all positively affected tissue TOL concentration. The precision obtained was relatively high ( $R^2 = 0.70$ ; CV = 16%).

250 ***Partial Least Squares (PLS) Regression***

251 Results of the PLS regression analysis are summarized in Table 3. When all four tissues were  
252 analyzed together, the variables retained in the best predictive model of TOL concentration  
253 were added VE (regression coefficient:  $0.63 \pm 0.14$ ), MUFA (regression coefficient:  $0.15 \pm$   
254  $0.38$ ) and dietary fat (regression coefficient:  $-0.13 \pm 0.36$ ). When the four tissues were  
255 analyzed individually, validated PLS models were only found for blood and muscle. For  
256 blood, added VE and MUFA were positively correlated with the TOL concentration, whereas  
257 dietary n-3 fatty acids were negatively correlated with the TOL concentration. In muscle,  
258 dietary levels of VE and n-6 fatty acids were positively associated with the TOL  
259 concentration.

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261 ***Data Partitioning***

262 Figure 2 represents the classification tree of the whole dataset, i.e., all four tissues analyzed  
263 together, into the different sets that best predicted the TOL concentration. Partitioning per  
264 tissue was not possible as there were not enough degrees of freedom to split further after the  
265 TOL dose effect. The overall standardized mean was  $0.75 \pm 1.29$ . The data set was divided  
266 into 2 subgroups, one with lower ( $0.36 \pm 0.72$ ) and one with greater ( $0.82 \pm 1.00$ )  
267 standardized tissue TOL concentration, defined by the added VE level, i.e., lower or greater  
268 than 40 mg/kg of diet, respectively. The second step of partition splits the data into 4 groups  
269 defined again by the added VE level:  $< 18.5$ ;  $[18.5; 40]$ ;  $[40; 50]$ ;  $> 50$  mg/kg). The third step  
270 of partition highlighted the effect of the type of dietary fatty acids on tissue TOL  
271 concentration. When added VE was  $< 18.5$  mg/kg of diet, SFA were the main factor  
272 influencing the TOL concentration with a critical threshold at 40.8% (below this  
273 concentration, the efficiency of SFA on TOL accumulation was about four times lower).  
274 When added VE was  $\geq 18.5$  mg/kg of diet, MUFA were the only factor affecting the TOL

275 concentration in tissues, with the highest TOL concentration obtained with the combination of  
276 MUFA > 35.4% and added VE  $\geq$  50 mg/kg of diet.

277 A summary of results obtained in the 3 statistical approaches is given in Table 4.

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## DISCUSSION

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The present study aimed to perform a meta-analysis, a tool commonly used in animal sciences (St-Pierre, 2007; Sauvant et al., 2008), to assess the potential relationship between the type of dietary fat and TOL concentration in pigs in the main VE storage tissues, namely blood, liver, muscle and adipose tissue.

The three statistical methods used in this study all demonstrated that added VE in feed was the main factor related to TOL concentration in tissues. Corino et al. (1999) reported an increase in tissue TOL concentration together with increasing VE dose, but the exact relationship, to our knowledge, has never been precisely established. Hasty et al. (2002) reported a linear relationship between muscle TOL concentration and dietary VE dose between 12 and 351 mg/kg of diet. In contrast, we showed a logarithmic dose-response relationship, described by an exponential model, with a maximum capacity of TOL accumulation similar to those also observed in pig muscles by previous authors (Jensen et al., 1997; Trefan et al., 2011). Sales and Koukolová (2011) also concluded from their meta-analysis that TOL concentration levelled out at 4.8 µg/g of muscle tissue. In the present study, the corresponding asymptote was found to be in the same range (4.6 µg/g of muscle tissue). Moreover, the greatest value of growth rate (i.e., the sensitivity of tissue to VE dose in feed) was observed in blood and the highest asymptote (corresponding to the highest storage capacity) was estimated for liver, confirmed that concentrations of TOL in plasma reflect more the immediate nutritional status of the animal (rapid turn-over) than adipose tissue and muscle, which reflect its long-term nutritional history. TOL deposition in tissues was different when VE added to feed was below or above 40 mg/kg, as suggested by results of the data partitioning. This dietary level, also estimated to be the minimum requirement for pigs (NRC,

304 2012), corresponds to the VE doses (40-50 mg/kg of diet) where the tissue TOL concentration  
305 started to plateau.

306 The three statistical approaches used in this study allow us to conclude that the  
307 quantity of added fat is not a factor that significantly affects the tissue TOL concentration, at  
308 least in the range of studied values in the meta-analysis. However, it is assumed that the  
309 digestion and absorption of VE is closely related to that of dietary fat (Thomson and Dietschy,  
310 1981; Borel et al., 2013) and it has been shown that dietary fat could enhance VE absorption  
311 in humans (Jeanes et al., 2004; Bruno et al., 2006). A first hypothesis to explain this finding is  
312 that the range of dietary fat used in the selected studies, i.e. between 0 and 10% (with a spot  
313 dose at 20% in Flachowsky et al., 1997), was not wide enough to find a significant effect of  
314 the dose of fat. Prola et al. (2013) also observed a 25% decrease in plasma TOL concentration  
315 with the use of 9% dietary fat (as soybean oil) as compared to the group receiving 4.5% fat.  
316 Therefore, a second hypothesis is that the effect of the dose of fat on VE bioavailability  
317 exhibits a bell curve with a maximum between 4.5 and 9% fat, which prevented us to observe  
318 a linear relationship between a fat dose below 20% and tissue VE concentration. A third  
319 hypothesis, although less likely knowing the observations in humans, is that the dose of  
320 dietary fat does not markedly affect VE bioavailability in pigs.

321 The main objective of this meta-analysis was to assess the effect of the type of dietary fatty  
322 acids on tissue VE concentration.

323 The main consistent finding was the effect of dietary MUFA. Indeed, all statistical analyses  
324 consistently showed that dietary MUFA were positively associated with tissue VE  
325 concentrations (Table 4). Furthermore, the partition analysis suggests that this association  
326 become significant when the VE content of the diet is above 18.5 mg/kg. We suggest this  
327 association might be due to the high oleic acid (C18:1) content of MUFA-rich diets. Indeed,  
328 once uptaken by the enterocyte, VE is secreted into chylomicrons, a step that is critically



329 dependent on the exogenous lipid supply and especially on the availability of oleic acid  
330 (Burton, 1994; Kayden and Traber, 1993; Anwar et al., 2007), the principal MUFA in fat  
331 sources. Therefore, MUFA (especially oleic acid), by facilitating the secretion of VE in  
332 chylomicrons, lead to a higher concentration of TOL in the blood and then in tissues.

333 Concerning the effect of SFA on TOL tissue deposition, both the results of the regression and  
334 partition analyses suggest that dietary SFA increase tissue TOL concentration. In support of  
335 this, SFA have recently been reported to allow a higher bioaccessibility of lipophilic  
336 micronutrient in vitro (Gleize et al., 2012). Nevertheless, the partition analysis suggests that  
337 this effect is most important at VE doses lower than 18.5 mg/kg of diet.

338 Another interesting relationship was that between dietary n-6 fatty acids and tissue VE  
339 deposition, which was observed both by regression and by PLS (in all tissues but also in  
340 muscle). This observation could be explained by the higher digestibility of these fatty acids in  
341 pigs compared to the other classes, as observed by Wiseman et al. (1991) and Powles et al.  
342 (1994). Indeed, VE is a highly lipophilic compound which is embedded in dietary fat and  
343 thus, a higher amount of VE would be released from the fat phase in the intestinal lumen in  
344 the presence of n-6-rich oil, leading to higher VE absorption. In addition, Yang and Mc  
345 Clements (2013) also demonstrated that corn oil (mainly composed of n-6 fatty acids) led to  
346 an increase in the solubilization capacity of VE into mixed micelles and therefore facilitated  
347 its conversion into free TOL, which may in turn affect the subsequent absorption of VE.

348 Finally, the PLS regression analysis revealed a negative association between diets rich in n-3  
349 fatty acids and blood TOL concentration. A decrease of plasma TOL concentration when the  
350 dietary content of n-3 fatty acids increased was also observed in rats (Tijburg et al., 1997) and  
351 in poultry (Villaverde et al., 2008). This was explained by a higher oxidative stress (Young et  
352 al., 2003) and consequently a higher lipid peroxidation process due to the presence of the very

353 oxidable n-3 fatty acids (Mahecha et al., 2010) which can lead to a higher VE mobilization  
354 and therefore a decrease in tissue TOL concentration.

355 Overall, it can be concluded from this meta-analysis that several dietary factors can modulate  
356 tissue TOL concentration in pigs. First of all, the VE dose in the diet is the main factor that  
357 influences tissue TOL concentration. Second, we observed a plateau in tissue TOL  
358 concentration at around 40-50 mg VE/kg of diet. Third, the amount of dietary fat, at least up  
359 to 20%, is not linearly correlated with tissue VE concentrations. Fourth, MUFA and SFA  
360 positively increase tissue TOL concentration when added VE is below 18.5 ppm for SFA and  
361 above 18.5 ppm for MUFA. Finally, we now have more certainties that the inclusion of n-3  
362 fatty acids in the diet provokes a decrease in tissue TOL concentration.

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## IMPLICATIONS

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366 Low inclusion level of vitamin E in the diet may cause several physiologic disorders being  
367 lethal for pigs in case of long-term deficiency. Vitamin E is also critical for the nutritional  
368 value and the organoleptic properties of meat, that is why it is important to optimize its  
369 supply. This study presented several complementary models that could be applied in practice  
370 to estimate the  $\alpha$ -tocopherol enrichment of pork meat according to the amount of  
371 supplemental dietary vitamin E and to the choice of fat source including in feed formulations.

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549

## TABLES AND FIGURES

550

551 **Table 1.** References used for meta-analysis of the relationship between dietary fat supplementation and TOL tissue concentration in pigs

Reference <sup>1</sup>	Nb pigs	Breed <sup>2</sup>	Gender	Added VE <sup>3</sup> (mg/kg)	Fat source <sup>4</sup> and quantity (%)	Fatty acid profile <sup>5</sup>	Duration of Supplementation (d)	Sampled tissues <sup>6</sup>
Malm et al., 1976	16	Y	gilt	0; 100	lard (8); corn oil (8)	estimated	110	serum
Monahan et al., 1992	64	L x LW	boar	10; 200	soya oil (3); tallow (3)	analyzed	n. p.	plasma, muscle, adipose tissue
Hidiroglou et al., 1993	27	n.p.	gilt	22; 44; 88	ctrl; tallow (5); fish oil (5)	estimated	71	plasma
Wang et al., 1996	20	L x LW	mix	0; 50	sunflower oil (1; 10); tallow (10)	estimated	56	plasma, liver, muscle, adipose tissue
Flachowsky et al., 1997	24	P x L x LW	mix	25; 37; 47	ctrl; rapeseed meal (10); soybean meal	analyzed	105	serum, liver, adipose tissue,

					(20)			muscle
Leskanich et al., 1997	150	NPD, PIC, JSR, C, N	mix	100; 250	tallow-soybean 4:1 (3); rapeseed oil-fish oil 2:1 (3) sunflower oil (2; 1.5+0.5 linseed oil); olive oil (2; 1.5+0.5 linseed oil)	analyzed	49	muscle, adipose tissue
Rey et al., 2001	80	L x LW	boar	10; 200		analyzed	42	muscle
Moreira and Mahan, 2002	248	(L x Y) x D	mix	0; 20; 40; 60	ctrl; CWG (5)	estimated	35	serum, liver, adipose tissue
Kouba et al., 2003	48	D x L x LW	gilt	150	ctrl; linseed (6) olive oil-lard (3); Sunflower oil-lard (3); palm oil-sunflower oil (3)	analyzed	60	muscle
López-Bote et al., 2003	60	L x GY	mix	20; 220		analyzed	n. p.	muscle
Guo et al., 2006	600	n.p.	mix	40; 200	NC (3.4); HOC (6.3);	estimated	63	plasma, muscle,

					HOC-CWG (10.1)			adipose tissue
Lauridsen, 2010	180	(L x Y) x D	mix	85; 150; 300	tallow (5); fish oil (5); sunflower (5)	analyzed	28	serum
Lauridsen et al., 2013	63	(L x Y) x D	mix	85; 150; 300	tallow (5); fish oil (5); sunflower (5)	analyzed	28	liver, adipose tissue

552 n. p.: not provided

553 <sup>1</sup>Peer-reviewed articles; chronologically sorted.

554 <sup>2</sup>Pig breed: C: Cotswold; D: Duroc; GY: Great York; JSR: JSR Genetics; L: Landrace; LW: Large White; N: Newsham Genetics LC;  
555 NPD: National Pig Development Company; P: Pietrain; PIC: Pig Improvement Company; Y: Yorkshire.

556 <sup>3</sup>Supplemented VE as dl-alpha-tocopheryl acetate. Reported values in the table are VE concentration theoretically added in the  
557 experimental diets. In the database used for statistical analyses, analytical values (when provided) were used.

558 <sup>4</sup>Fat sources: ctrl: experimental diet with no added fat; CWG: choice white grease; HOC: high oil corn; NC: normal corn.

559 <sup>5</sup>Determination of the fatty acids composition of experimental diets; “analyzed”: the authors performed the lab analysis and published it in  
560 the paper; “estimated”: when not available, the fatty acids profile of the diets was estimated using the NRC 2012 and the NRC 1998 (for n-3 and  
561 n-6 values).

562 <sup>6</sup>Muscle referred to longissimus dorsi, thoracis or lumborum and adipose tissue to backfat.

563

564 **Table 2.** Exponential model parameters used to describe the relationship between vitamin E dose and tissue  $\alpha$ -tocopherol concentration<sup>1</sup>

565

Tissues	Asymptote (a)		Scale (b)		Growth Rate (c)	
	Estimate	95% Confidence	Estimate	95% Confidence	Estimate	95% Confidence
	$\pm$ SE	Interval	$\pm$ SE	Interval	$\pm$ SE	Interval
Blood	2.14 $\pm$ 0.51	[1.13; 3.14]	-1.26 $\pm$ 0.91	[-3.05; 0.52]	-0.09 $\pm$ 0.20	[-0.47; 0.30]
Muscle	4.49 $\pm$ 0.51	[3.49; 5.49]	-3.45 $\pm$ 0.94	[-5.30; -1.61]	-0.04 $\pm$ 0.03	[-0.09; 0.01]
Liver	15.91 $\pm$ 21.74	[-26.70; 58.51]	-14.7 $\pm$ 21.56	[-56.96; 27.56]	-0.01 $\pm$ 0.03	[-0.06; 0.04]
Adipose tissue	8.66 $\pm$ 0.72	[7.25; 10.07]	-6.36 $\pm$ 1.11	[-8.54; -4.18]	-0.05 $\pm$ 0.03	[-0.1; 0.00]

566

567 <sup>1</sup>The exponential model employed is  $Y = a + b \cdot \exp^{(c \cdot X)}$ , where “Y” is the tissue  $\alpha$ -tocopherol concentration ( $\mu\text{g/g}$  or  $\mu\text{g/ml}$ ), “X” is the  
568 vitamin E added in feed ( $\text{mg/kg}$ ), “a” is the asymptote (tissue  $\alpha$ -tocopherol concentration when the vitamin E dose in feed tends towards  $\infty$ ), “b”  
569 is the scale (difference between baseline value ( $X = 0$ ) and asymptote), and “c” is the growth rate (slope of the linear curve before reaching the  
570 asymptote).

571 **Table 3.** Outputs of the Partial Least Square (PLS) regression analysis performed to explain the variance of the TOL tissue concentration<sup>1</sup>

572

Tissue <sup>2</sup>	Nb of replications	R <sup>2</sup>	Q <sup>2</sup>	Variable retained in the final model (regression coefficient value)
All tissues <sup>3</sup>	112	64.0%	27.8%	VE (0.63); MUFA (0.15); fat (-0.13)
Blood <sup>4</sup>	27	54.5%	37.5%	VE (0.73); MUFA (0.18); n-3 (-0.19)
Muscle	34	55.9%	48.4%	VE (0.74); n-6 (0.16)

573 <sup>1</sup>Different PLS regression models were built using increasing Variable Importance in the Projection threshold values. The X-variables  
574 included in the PLS regression analysis were: supplemented vitamin E in feed (VE); dietary fat; saturated fatty acids; monounsaturated fatty  
575 acids (MUFA); polyunsaturated fatty acids; omega-3 polyunsaturated fatty acids (n-3); omega-6 polyunsaturated fatty acids (n-6). The Y  
576 variable was the standardized tissue TOL concentration.

577 <sup>2</sup>No validated PLS regression model was found to explain the variance of the TOL concentration individually in liver (n = 18) and in  
578 adipose tissue (n = 33).

579 <sup>3</sup>The analysis was carried out with pooled and normalized data from blood, muscle, liver and adipose tissue together.

580 <sup>4</sup>Concentrations measured in plasma and serum.

581

582 **Table 4.** Relationship between dietary fatty acids and tissue TOL concentration in pigs. Summary of 3 different statistical analyses

Methods <sup>1</sup>	added	added	Type of fatty acids <sup>4</sup>				
	VE <sup>2</sup>	fat <sup>3</sup>	SFA	MUFA	PUFA	n-3	n-6
Regression	++	ns	+	+	ns	ns	+
PLS	++	-	ns	+	ns	-	+
	blood/muscle			blood		blood	muscle
Partitioning	++	ns	+	+	ns	ns	ns

583 Effects (positive or negative) are given for all tissues (muscle, liver, blood, adipose tissue) unless it is specified. ns: non significant  
 584 relationship between the independent variable and tissue TOL concentration.

585 <sup>1</sup>Regression (stepwise regression; exponential model); PLS: Partial Least Squares regression; Partitioning: Partition analysis.

586 <sup>2</sup>Supplementation of vitamin E (as dl- $\alpha$ -tocopheryl acetate) to the diet.

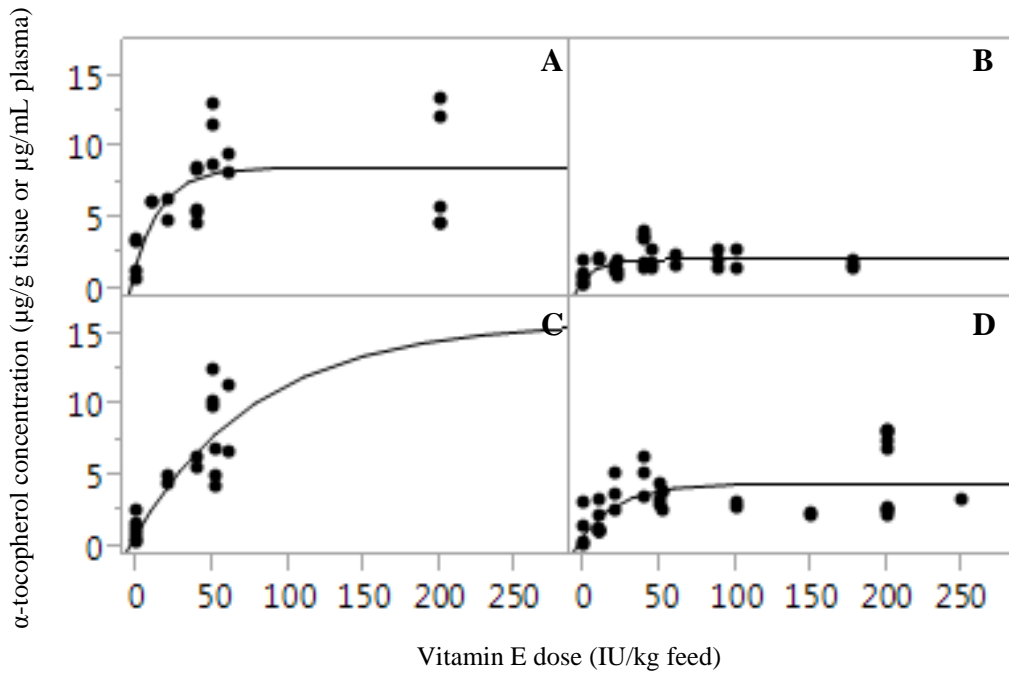
587 <sup>3</sup>Supplementation of fat source to the diet.

588 <sup>4</sup>SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; n-3: omega-3 fatty acids; n-6:  
 589 omega-3 fatty acids.



590 **Figure 1.**  $\alpha$ -tocopherol concentrations in pig tissues as a function of the dose of vitamin E in  
591 diet<sup>1</sup>

592



593

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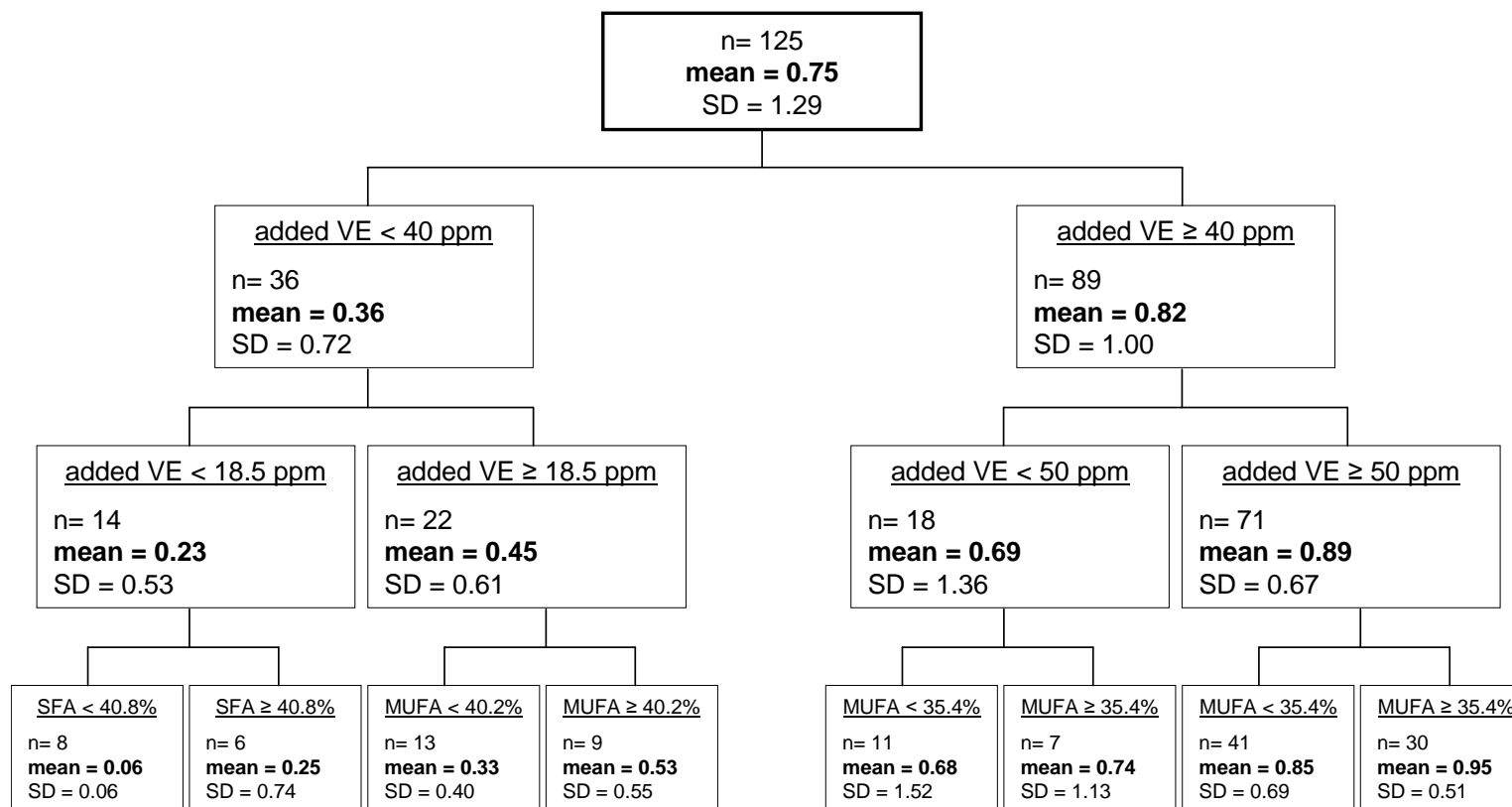
595

596 <sup>1</sup>Data from 13 different studies. The exponential model parameters used to describe  
597 the relationship between vitamin E dose and tissue  $\alpha$ -tocopherol concentration are shown in  
598 Table 2.

599 Pig tissues: A: adipose tissue; B: blood (compilation of plasma and serum values); C:  
600 liver; D: muscle.

601

602 **Figure 2.** Classification tree of data partitioning<sup>1</sup>



603

604 <sup>1</sup>The partition algorithm recursively partitions data according to a relationship between the X (explicative variables) and the Y values  
 605 (normalized  $\alpha$ -tocopherol concentrations in pooled tissues), creating a tree of partitions. It finds a set of cuts or groupings of X values that best  
 606 predict a Y value. A group of X values is characterized by its mean value (standardized TOL concentration), its standard deviation (SD) and the  
 607 number of replication (n).

608           The 3 explicative variables that significantly explain the variance: added VE: VE dose in diet (in mg/kg); SFA: saturated fatty acids;  
609   MUFA: monounsaturated fatty acids.

