

# INTERACTION BETWEEN DIETARY UNSATURATION AND $\alpha$ -TOCOPHEROL LEVELS: VITAMIN E CONTENT IN THIGH MEAT

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## Abstract

The present study was carried out to evaluate the influence of dietary polyunsaturated fatty acids (PUFA), at different levels of dietary supplementation with  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA), on  $\alpha$ -tocopherol ( $\alpha$ -Toc) content in raw thigh meat. One hundred and ninety-two female broiler chickens were randomly distributed into 16 experimental treatments resulting from the combination of 4 levels of dietary polyunsaturated fatty acids (27, 38, 48 and 59 g/kg) and 4 levels of supplementation with  $\alpha$ -TA (0, 100, 200 and 400 mg/kg). As is to be expected, dietary supplementation with  $\alpha$ -TA increased  $\alpha$ -Toc content of thigh meat. But the vitamin E enrichment magnitude in meat depends on the dietary PUFA content ( $P \leq 0.001$ ). Furthermore,  $\alpha$ -Toc content of thigh meat was reduced as the inclusion of dietary PUFA increased. Thus, in treatments supplemented with 100 mg  $\alpha$ -TA/kg, an increase of 32 g dietary PUFA significantly reduced  $\alpha$ -Toc content of meat by 52%.

## Résumé

Cette expérience a été conduite pour évaluer l'influence des acides gras polyinsaturés alimentaires (PUFA), avec différents niveaux d'acétate de  $\alpha$ -tocophérol ( $\alpha$ -TA), sur le contenu de  $\alpha$ -tocophérol ( $\alpha$ -Toc) de la viande crue de la cuisse. Cent quatre-vingt douze femelles de poulet de chair ont été réparties au hasard dans 16 traitements expérimentaux correspondant à 4 niveaux d'acides gras polyinsaturés alimentaires (27, 38, 48 et 59 g/kg) et 4 niveaux de supplémentation en  $\alpha$ -TA (0, 100, 200 et 400 mg/kg). La supplémentation du régime en  $\alpha$ -TA augmente le contenu de  $\alpha$ -Toc de la viande de la cuisse. Mais l'importance de l'enrichissement en vitamine E dans la viande dépend du contenu des PUFA dans la diète ( $P < 0.001$ ). De plus, le contenu en  $\alpha$ -Toc dans la viande de la cuisse est diminué quand l'apport des PUFA dans la diète

augmente. Ainsi, dans les traitements supplémentés avec 100 mg  $\alpha$ -TA/kg, une augmentation de 32 g des PUFA dans le régime réduit le contenu de  $\alpha$ -Toc de la viande de 52%.

## Introduction

There is a trend towards rising unsaturation degree in chicken meat for a double reason. On the one hand, human nutritive recommendations suggest reducing saturated fatty acids intake, and on the other hand, the use of animal fats has been reduced in Europe. The higher unsaturation degree of meat produces organoleptic detriment (Bou et al., 2001) and leads to a major susceptibility to lipid oxidation (Grau et al., 2001). It is well established that dietary  $\alpha$ -Toc supplementation prevents lipid oxidation (De Winne and Dirinck, 1996; Grau et al., 2001) and increases vitamin E content in poultry meat (Grau et al., 2001). Nevertheless, there are few data regarding the balance between dietary PUFA and vitamin E to reach nutritional benefits in meat.

## Material and Methods

### *Animals and diets*

One hundred and ninety-two female broiler chickens at one day of age were randomly distributed into 16 dietary treatments of three replicates each. Broilers were kindly supplied by Terra-Avant S.A. (Girona, Spain). The animals were housed in groups of four in forty-eight cages under standard conditions of temperature, humidity and ventilation.

The diets were formulated according to exceed the requirements recommended by the NRC (1994) on the basis of maize and soya. The experimental treatments were resulting from the combination of 4 levels of dietary polyunsaturated fatty acids: 27, 38, 48 and 59 g/kg and 4 levels of supplementation with  $\alpha$ -TA: 0, 100, 200 and 400 mg/kg. The unsaturation degree was achieved by adding increasing proportions (2, 4, 6 and 8%) of a linseed and fish oil mixture (ratio 4:1) (Table 1).

Feed and water were provided ad libitum during the 40 days of experimental period. Feed samples were taken three times during the experiment for  $\alpha$ -Toc content determination.

### *Sample collection*

At the end of the experimental period, two animals per cage were killed in a commercial slaughterhouse. Thigh muscles were removed and weighed individually.

Thighs were boned and ground with skin. Samples were freeze-dried, ground and stored at  $-20^{\circ}\text{C}$  until further analyses.

#### *$\alpha$ -Tocopherol analysis*

$\alpha$ -Toc from feeds and thigh was extracted as described previously by Jensen et al. (1999) starting from 2 g of feeds and 100 mg of freeze-dried sample.

Table 1. Composition of experimental diets.

Ingredients (%)	Dietary unsaturation (g/kg) <sup>4</sup>			
	27	38	48	59
Maize	58.49	52.68	46.86	41.02
Soya 48	35.49	36.42	37.34	38.24
Added oil <sup>1</sup>	2.00	4.00	6.00	8.00
Almond husk	0.00	2.92	5.84	8.76
Dicalcium phosphate	1.75	1.76	1.76	1.77
Calcium carbonate	1.08	1.07	1.06	1.05
Salt	0.57	0.57	0.57	0.57
Vitamin mineral mix <sup>2</sup>	0.40	0.40	0.40	0.40
DL-Methionine	0.18	0.19	0.19	0.20
L-Lysine	0.05	0.03	0.02	0.00
<b>Chemical analysis (%)</b>				
Dry matter	88.26	89.08	89.42	90.28
Crude protein	21.31	21.82	21.48	21.85
Crude fat	4.45	6.30	8.40	9.91
Ash	5.79	6.10	5.85	6.20
ME (Kcal/kg) <sup>3</sup>	3000	3003	3006	3007

<sup>1</sup>Linseed and fish oil mixture in a ratio 4:1.

<sup>2</sup>Vitamin and mineral mix per kg of feed: Vitamin A: 12000 UI; Vitamin D<sub>3</sub>: 2400 UI; Vitamin K<sub>3</sub>: 3 mg; Vitamin B<sub>1</sub>: 2.2 mg; Vitamin B<sub>2</sub>: 8 mg; Vitamin B<sub>6</sub>: 5 mg; Vitamin B<sub>12</sub>: 11  $\mu\text{g}$ ; Folic acid: 1.5 mg; Biotin: 150  $\mu\text{g}$ ; Calcium pantothenate: 25 mg; Nicotinic acid: 65 mg; Mn: 60 mg; Zn: 40 mg; I: 0.33 mg; Fe: 80 mg; Cu: 8 mg; Se: 0.15 mg.

<sup>3</sup>Estimated values.

<sup>4</sup>Each level of unsaturation was supplemented with four different levels of  $\alpha$ -TA (0, 100, 200 and 400 mg/kg).

#### *Statistics*

ANOVA was carried out to determine whether the factors studied (dietary unsaturation and  $\alpha$ -TA level) affected  $\alpha$ -Toc content in thigh meat. In all cases,  $P \leq 0.05$  was considered significant.

## Results and discussion

The  $\alpha$ -Toc content of raw thigh, expressed as mg/kg of thigh is shown in Table 2. Dietary supplementation with  $\alpha$ -TA and dietary unsaturation level had significant effect ( $P \leq 0.0001$ ) on the  $\alpha$ -Toc content in thigh.

$\alpha$ -Toc content of thigh significantly increased with the dietary supplementation with  $\alpha$ -TA. Several authors have reported that  $\alpha$ -Toc content in poultry tissues increases linearly with dietary supplementation (Jensen et al., 1999; Cortinas et al., 2001). However, there is a wide range of variability in the  $\alpha$ -Toc content of chicken meat obtained by different authors with similar levels of  $\alpha$ -TA supplementation. This variation can be due in part to the studied tissue, and mainly to the lipid composition of the meat, depending on amount and profile.

Table 2. Effect of dietary unsaturation and  $\alpha$ -tocopheryl acetate supplementation on  $\alpha$ -tocopherol content in thigh meat (expressed as mg/kg thigh)<sup>1</sup>.

Dietary PUFA (mg/kg)	Global Mean	$\alpha$ -Tocopheryl Acetate (mg/kg)			
		0	100	200	400
27	23.17 <sup>x</sup>	0.33 <sup>g</sup>	12.57 <sup>ef</sup>	24.34 <sup>cd</sup>	55.46 <sup>a</sup>
38	18.48 <sup>y</sup>	0.43 <sup>g</sup>	12.89 <sup>ef</sup>	19.05 <sup>de</sup>	41.56 <sup>b</sup>
48	14.63 <sup>z</sup>	0.10 <sup>g</sup>	10.34 <sup>ef</sup>	17.57 <sup>de</sup>	30.52 <sup>c</sup>
59	12.93 <sup>z</sup>	0.04 <sup>g</sup>	6.05 <sup>fg</sup>	14.98 <sup>ef</sup>	30.64 <sup>c</sup>
<b>Global Mean</b>		0.22 <sup>z</sup>	10.46 <sup>y</sup>	18.98 <sup>x</sup>	39.55 <sup>w</sup>
<b>SEM</b>	1.840				
<b>P values</b>					
Unsaturation		0.0001			
$\alpha$ -TA		0.0001			
Unsaturation $\times$ $\alpha$ -TA		0.0001			

<sup>1</sup>Values given in this table correspondence to least-square means obtained from ANOVA (n=6) and their pooled SEM.  $\alpha$ -TA:  $\alpha$ -Tocopheryl acetate.

<sup>a,b,c,d,e,f,g</sup>: Different superscripts indicate significant differences in the interaction between dietary unsaturation and  $\alpha$ -tocopheryl acetate supplementation.

<sup>w,x,y,z</sup>: Different superscripts indicate significant differences in the global means.

At a dietary levels of 200 mg  $\alpha$ -TA/kg approx., Grau et al. (2001) found higher  $\alpha$ -Toc content in thighs with skin (from 21.8 to 25.2 mg  $\alpha$ -Toc/kg) than those (De Winne and Dirinck, 1996; Maraschiello et al., 1999; Ruiz et al., 1999) in thighs without skin and therefore lower lipid content (from 8.7 to 17.5 mg  $\alpha$ -Toc/kg).

But probably the more important factor which causes the main differences among works are those related to the PUFA composition of the diet. The present work shows that dietary unsaturation affected the  $\alpha$ -Toc deposit in thigh. As such,  $\alpha$ -Toc content of thigh was reduced as the inclusion of dietary PUFA increased. Thus, an increase of 32 g dietary PUFA significantly reduced  $\alpha$ -Toc content of meat by 52, 38 and 45% in treatments supplemented with 100, 200 and 400 mg  $\alpha$ -TA/kg, respectively ( $P \leq 0.05$ ). In literature studies which use fat and oil sources varying in the unsaturation degree found variable results concerning  $\alpha$ -Toc accumulation in chicken meat.

Dietary unsaturation gives rise to higher content of PUFA in chicken meat, which has a higher oxidative status (Grau et al., 2001). The relative quantities of  $\alpha$ -Toc required to protect fatty acid are higher as double bounds in the molecule of the fatty acid increase (Witting and Horwitt, 1964). For that reason, studies adding to diets fish oil rich in long chain PUFA with a high number of double bounds found a reduction in the  $\alpha$ -Toc content in chicken tissues (Miller and Huang, 1993; Husveth et al., 2000; Surai and Sparks, 2000; Cortinas et al., 2001). However, authors who used dietary vegetable oils with high levels of PUFA with a lower number of double bounds, did not find differences in  $\alpha$ -Toc content in chicken tissues (Cherian et al., 1996; Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001).

In conclusion, it is important to know the amount of PUFA, as well as the degree of unsaturation of PUFA of diets to optimise and to adjust the amount of  $\alpha$ -TA supplemented in chicken feeds.

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