

# $\alpha$ -TOCOPHEROL CONTENT AND LIPID OXIDATION IN FRESH, COOKED AND SCRAMBLED EGGS ENRICHED WITH $\omega$ -3 FATTY ACIDS.

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## Summary

The present study was carried out to evaluate the effect of dietary supplementation with  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA) on  $\alpha$ -tocopherol ( $\alpha$ -Toc) content and lipid oxidation (TBA values) in fresh, cooked and scrambled eggs enriched with  $\omega$ -3 fatty acids. Four treatments were formulated from a basal diet containing 4% linseed oil (L) or fish oil (F) with 0 or 100 mg of  $\alpha$ -TA/kg of feed. Dietary supplementation with 100 mg/kg  $\alpha$ -TA significantly increased  $\alpha$ -Toc content of eggs. Fresh, cooked and scrambled eggs from F treatments showed lower  $\alpha$ -Toc content than those from L and their processing significantly decreased the  $\alpha$ -Toc content. Moreover, processing of eggs significantly increase lipid oxidation. TBA values in cooked and scrambled eggs were significantly reduced when 100 mg  $\alpha$ -TA /kg of feed were supplemented to the diet. Reduction of TBA values caused by  $\alpha$ -TA supplementation was more pronounced in eggs from F treatments (0.72% C20:5  $\omega$ 3; 7.15% C22:6  $\omega$ 3) than in those from L (8.27% C18:3  $\omega$ 3).

**Key words:**  $\alpha$ -tocopherol, lipid oxidation, processing  $\omega$ 3-PUFA enriched eggs

## Introduction

Over recent years the enrichment of eggs with  $\omega$ 3 polyunsaturated fatty acids (PUFA) has been associated with prevention of cardiovascular diseases. The higher PUFA content of these eggs leads to an increase in their unsaturation and, thus, to a major susceptibility to lipid oxidation. To prevent lipid oxidation in those products, dietary supplementation with tocopherols have been effectively used (Galobart *et al.*, 2001). Moreover, this supplementation produce eggs enriched with vitamin E. Although it has been demonstrated that processing of eggs increase their oxidation, there are few data regarding the effect of processing eggs enriched with  $\omega$ 3 PUFA on the  $\alpha$ -Toc content of eggs.

The objective of the present study was to determine the effect of different cooking processes (hard boiling and scrambling) on the  $\alpha$ -Toc content and lipid oxidation in eggs enriched with  $\omega$ 3 PUFA [linolenic acid (LNA) or very long chain  $\omega$ 3 PUFA].

## Materials and methods

Forty LSL-White Leghorn hens at 20 weeks of age were randomly distributed into four treatments. Diets were formulated from a basal diet containing 4 % linseed oil (L) (39.2% LNA) or fish oil (F) (25.6% C20:5  $\omega$ 3 + C22:6  $\omega$ 3) with 0 or 100 mg of  $\alpha$ -TA/kg of feed.

After 25 days of feeding, 30 eggs were collected from each dietary treatment group and for each determination (whole fresh, cooked and scrambled eggs). Eggs from each treatment were distributed into 6 samples of 5 pooled eggs. Samples were analysed for  $\alpha$ -Toc content and lipid oxidation.

Eggs from each dietary treatment were hard cooked at 100°C during 30 min. For scrambling, eggs were homogenized and cooked in a pan on an electric stove until a final internal temperature of 80°C was reached.

$\alpha$ -Toc from whole fresh, cooked and scrambled eggs (5 samples per treatment) was extracted as described by Abdollahi *et al.* (1993), and HPLC determination was performed according to Drotleff and Ternes (1999). TBA values in fresh, cooked and scrambled eggs (6 samples per

treatment) were analysed as described by Grau *et al.* (2000), starting from 1.5 g of sample.

Multifactor ANOVA were carried out to determine whether the factors studied (oils,  $\alpha$ -TA level and processing) affected  $\alpha$ -Toc content and lipid oxidation in fresh, cooked and scrambled eggs. In all cases, P values  $\leq 0.05$  were considered significant.

## Results and discussion

$\alpha$ -Toc level in the diets were 29, 96.5, 34 and 117 mg  $\alpha$ -Toc/kg of feed for L, L +  $\alpha$ -TA, F and F +  $\alpha$ -TA, respectively.

### $\alpha$ -Tocopherol content

Eggs from treatments supplemented with 100 mg/kg  $\alpha$ -TA of feed showed higher  $\alpha$ -Toc levels than those from not-supplemented groups (Table 1). Several authors have reported that  $\alpha$ -Toc content in fresh eggs increased linearly with dietary  $\alpha$ -TA supplementation (Meluzzi *et al.*, 2000; Galobart *et al.*, 2001). However, there is a wide range of variability in the  $\alpha$ -Toc content of eggs obtained by others authors with similar levels of  $\alpha$ -TA supplementation. This variation can be due in part to the analytical methods used to determine  $\alpha$ -Toc in the feed and eggs, and to the fact that the actual  $\alpha$ -Toc content in the diet do not always coincide with the estimated  $\alpha$ -Toc from dietary supplementation. But probably the more important factor which causes the main differences among works are those related to the lipid composition of diet (amount and profile). In fact, dietary oils had effect on  $\alpha$ -Toc deposit in eggs. So,  $\alpha$ -Toc content in eggs was lower in those from hens fed diets with F than those from fed diets with L. Similarly, other authors found that  $\alpha$ -Toc content was lower in those treatments with a higher level of very long chain  $\omega$ 3 PUFA (Meluzzi *et al.*, 2000; Galobart *et al.*, 2001), and hypothesize a possible interference between them in the intestinal absorption.

There are few studies that have dealt with the variation in the  $\alpha$ -Toc content of eggs caused by cooking processes. In our study, the effect of processing depended among dietary oil. So,  $\alpha$ -Toc content was reduced by 12 and 16% when eggs from F treatments were cooked and scrambled,

respectively, but no effect was observed in eggs from L treatments (Table 1).

**Table 1.** Effect of dietary oils,  $\alpha$ -tocopheryl supplementation and cooking process on the  $\alpha$ -tocopherol content in fresh, cooked and scrambled eggs ( $\mu\text{g/g}$  eggs solids).

Global Mean	Oil		$\alpha$ -Tocopheryl acetate (mg/kg)	
	Linseed	Fish	0	100
(n=20)	(n=10)	(n=10)	(n=10)	(n=10)
Fresh	297.54	321.57 <sup>a</sup>	273.5 <sup>b</sup>	187.17
Cooked	282.92	325.23 <sup>a</sup>	240.62 <sup>c</sup>	178.33
Scrambled	283.35	336.86 <sup>a</sup>	229.84 <sup>c</sup>	194.79
Global Mean		327.89	247.99	186.77
SEM			9.981	389.11
P values				
Oil	0.0001	Oil $\times$ $\alpha$ -TA	0.9441	
$\alpha$ -TA	0.0001	Oil $\times$ Processing	0.0170	
Processing	0.2663	$\alpha$ -TA $\times$ Processing	0.1245	

<sup>a,b,c</sup> Different superscripts indicate significant differences in the interaction Oil  $\times$  Processing.  $\alpha$ -TA = supplementation with 100 mg/kg  $\alpha$ -tocopheryl acetate

Murcia et al. (1999) observed that  $\alpha$ -Toc content of egg yolk was reduced by 20% in yolks cooked during 3 or 10 min, and by 50% in yolks processed as omelette or after heating in a microwave oven. Differences observed between our study and that by Murcia et al. (1999) may be attributed to the fact that in our case, whole eggs had a higher water content than egg yolk. According to Yoshida et al. (1988) a high water content in a food may protect the triglycerides from lipid oxidation during cooking processes.

#### Lipid oxidation

In fresh eggs, there were not differences in TBA values for any of the studied factors, with values ranging from 30 to 35 ng malondialdehyde (MDA)/g egg (Table 2).

**Table 2.** Effect of dietary oils,  $\alpha$ -tocopheryl acetate supplementation and processing on TBA values in fresh, cooked and scrambled eggs (expressed as ng MDA/g egg).

Treatment <sup>1</sup>	Processing		
	Fresh	Cooked	Scrambled
L	31.45	129.44	101.49
L + $\alpha$ -TA	32.45	98.89	94.19
F	30.59	289.19	231.36
F + $\alpha$ -TA	34.58	173.3	127.36
SEM		27.128	
P values			
Oil	0.0001	Oil $\times$ $\alpha$ -TA	0.0001
$\alpha$ -TA	0.0001	Oil $\times$ Processing	0.0001
Processing	0.0001	$\alpha$ -TA $\times$ Processing	0.0002

<sup>1</sup>L= Linseed oil; F = Fish oil;  $\alpha$ -TA = supplementation with 100 mg/kg  $\alpha$ -tocopheryl acetate

Cooking and scrambling of eggs increased 3 to 10 fold TBA values of fresh eggs. In addition, dietary oils affected TBA values in cooked and scrambled eggs. Thus, lipid oxidation in cooked and scrambled eggs from F treatments were 45% higher than those from L treatments ( $P \leq 0.0001$ ). This higher susceptibility to lipid oxidation of eggs from F treatments may be attributed to its higher content in very long chain  $\omega$ 3 PUFA. Other authors found that oxidative stability of eggs decreases as unsaturation level ( $\omega$ 3 vs.  $\omega$ 6) of eggs increases (Grashorn and Steinhilber, 1999; Galobart et al., 2001).

Although  $\alpha$ -TA supplementation did not modify TBA values in fresh eggs, when eggs were cooked and scrambled lipid oxidation was reduced by 35.0% and 33.7% by addition of 100 mg  $\alpha$ -TA/kg, respectively. Besides, an interaction between  $\alpha$ -TA supplementation and dietary oil was observed in the sense that  $\alpha$ -TA supplementation prevented lipid oxidation more effectively in eggs from F treatments than those from L. It confirms that eggs from F diets show higher susceptibility to oxidation and they require higher contribution of  $\alpha$ -Toc, so a lower level of  $\alpha$ -Toc in eggs from F treatments was observed (Table 1). There are few studies dealing with the effect of processing eggs on lipid oxidation. In our case, cooked eggs showed TBA values higher than scrambled eggs. Some authors found that spray-drying of eggs significantly increase lipid oxidation (Galobart et al., 2001).

From our results and those from other researches we can conclude that fatty acid composition of diets and processing of eggs affect  $\alpha$ -tocopherol content and lipid oxidation of such products, and thus, dietary supplementation with  $\alpha$ -TA could be adjusted depending on these factors.

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