

## VITAMIN E LEVELS AND LIPID OXIDATION IN $\omega$ 3 FATTY ACIDS ENRICHED EGGS.

J. Galobart<sup>1</sup>; A.C. Barroeta<sup>1</sup>; M.D. Baucells<sup>1</sup> and F. Guardiola<sup>2</sup>

<sup>1</sup>UD Nutrició i Alimentació Animals. Universitat Autònoma de Barcelona. 08193 Bellaterra. Spain. E-mail: [Ana.Barroeta@uab.es](mailto:Ana.Barroeta@uab.es)

<sup>2</sup>Dep. Ciències Fisiològiques Humanes i de la Nutrició. Universitat de Barcelona. Av. Joan XXIII, 08028 Barcelona. Spain.

### Summary

With the aim to establish the required amount of  $\alpha$ -tocopherol to effectively prevent lipid oxidation in  $\omega$ 3 FA enriched eggs, a trial was conducted with 288 laying hens. Birds were randomly assigned to four treatments resulted from supplying a basal diet containing 5% of linseed oil (diet: 65% PUFA, 34,5%  $\omega$ 3) with 0 (C), 50 (50E), 100 (100E) or 200 (200E) ppm of alpha-tocopherol acetate. After 25 days of feeding, eggs were collected and lipid oxidation was measured by the Induced TBARS methodology (Kornbrust and Mavis, 1980) and expressed as ng MDA/g egg.

At 150 min of incubation, supplementation with 50, 100, or 200 ppm of Vitamin E promoted a dramatic decrease in the TBARS values of all treatments when compared to the control group (C: 2,63 vs. 50E: 0,47; 100E: 0,48; 200E: 0,38;  $P < 0.001$ ). Moreover, no significant differences were found according to different levels of Vitamin E supplementation.

From these data, we can conclude that supplying hens' diets with 50 ppm of alpha-tocopherol acetate can effectively reduce the lipid oxidation in  $\omega$ 3 FA enriched eggs.

### Introduction

In avians, dietary supplementation with  $\alpha$ -tocopherol has been reported to increase  $\alpha$ -tocopherol content of hen eggs in a dose-dependent manner (Wahle et al, 1993; Jiang et al, 1994; Surai et al, 1995).

Supplementation with vitamin E is very important to avoid lipid oxidation in  $\omega$ 3-PUFA enriched products like eggs or meat. In the literature there are some works that show a protective effect of supplementing hen diet with high amounts of vitamin E (more than 200 ppm) on egg lipid oxidation (Noel et al, 1996; Cherian et al, 1996; Galobart et al, 1998), but there are few works studying the effect of graded levels of vitamin E in spray-dried eggs (Wahle et al, 1993) and fresh eggs (Qi and Sim, 1998).

- Due to the high cost of supplementing the diet with vitamin E, it is very important to establish the minimum levels of  $\alpha$ -tocopherol needed in diets not only to effectively prevent lipid oxidation in animal products but also to be acceptable economically.

The objective of the present study is to establish the minimum amount of vitamin E necessary to prevent lipid oxidation in  $\omega$ 3 FA enriched eggs.

## Materials and methods

288 Lohman laying hens were randomly assigned to 4 dietary treatments (12 replicates/treatment 6 hens/replicate). Experimental treatments were formulated to meet NRC (1994) requirements and consisted on a basal diet containing 5% Linseed oil (65% PUFA, 34,5%  $\omega$ 3) unsupplemented (C) or supplemented with 50 (50E), 100 (100E) or 200 (200E) ppm of alpha-tocopheryl acetate (Roche Vitaminas, Madrid, Spain).

After 25 days of feeding, all eggs produced in each replicate were collected during 4 days and held at 4 °C. After this time, 5 eggs of each replicate were cracked, homogenised and frozen at -80 °C until their analyses for lipid oxidation.

Lipid oxidation was measured in 12 samples (pool of 5 eggs) per treatment, according to the Induced TBARS methodology (Kornbrust and Mavis, 1980) with some modifications. 5 mL of a solution of 30% egg in KCl (1.15%) was incubated at 37 °C in 25 mL of tris-malate tampon 80 mM, 10 mL ascorbic acid 2 mM and 10 mL ferrous sulphate 5 mM. At 0, 20, 40, 60, 90 and 150 minutes 2 mL of the incubate were mixed with 4 mL of TBA-TCA-HCL and after 15 min in boiling water, absorbance at 531 nm was read. Results are expressed in ng MDA/g egg.

All data were analysed by ANOVA using the SAS<sup>®</sup> General Linear Models procedures (SAS Institute, 1996).

## Results and discussion

Susceptibility to lipid oxidation is shown in table 1 and figure 1, expressed as ng MDA/g egg.

During all incubation time, the control group showed higher TBARS values than the vitamin E supplemented groups. At 150 minutes of incubation TBARS supplementation with 50, 100 or 200 ppm promoted a dramatic decrease in lipid oxidation compared to the non-supplemented group (2,63 vs. 0,47; 0,48; 0,38; for C, 50E, 100E and 200 E, respectively,  $p < 0,001$ ). Moreover, no significant differences were observed between supplementing with 50, 100 or 200 ppm of  $\alpha$ -tocopherol.

Our results are comparable with those obtained by Wahle et al (1993) who, using similar vitamin E levels to our study in spray-dried eggs, found that egg powders obtained from hens without vitamin E supplementation had higher TBA values than those from hens fed graded levels of vitamin E (25, 50, 75, 100 and 200 ppm). Only small differences were found among the different levels of vitamin E supplementation. Differences among levels became apparent at 8-12 months of storage, and TBA values were inversely related to vitamin E content of eggs. Storage led to a progressive decline in vitamin E content of the eggs. So, differences between treatments will appear when vitamin E concentration in the egg is too low to avoid initiation and extension of lipid oxidation.

Contrasting our results of induced oxidation and those obtained by Wahle et al (1993) in spray-dried eggs, (where eggs are subjected to prooxidant conditions), Qi and Sim (1998) found significant reduction in TBA values in fresh eggs when supplemented hens' diet with 200 or 400 ppm of vitamin E. Direct comparison of the results is complex because of the different methodologies used for determining lipid oxidation, and also for the different levels of vitamin E used.

However, Vitamin E content of eggs decrease with processing and storage time. Thus, Vitamin E levels required in hens diet should be adjusted depending not only on the fatty acid composition of the eggs (directly related to the diet), but also on the conditions of processing and conditions and time of storage.

Further studies on evolution of vitamin E concentration and lipid oxidation during processing and storage under different conditions or periods of time are required to adjust the minimum amount of vitamin E required in hens diet.

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Table 1. Evolution of TBARS values expressed as ng MDA/g egg.

Time (min.)	0	20	40	60	90	150
C	0,62 <sup>a</sup>	1,30 <sup>a</sup>	2,24 <sup>a</sup>	2,57 <sup>a</sup>	2,62 <sup>a</sup>	2,63 <sup>a</sup>
50E	0,49 <sup>ab</sup>	0,54 <sup>b</sup>	0,45 <sup>b</sup>	0,46 <sup>b</sup>	0,49 <sup>b</sup>	0,47 <sup>b</sup>
100E	0,42 <sup>b</sup>	0,44 <sup>b</sup>	0,42 <sup>b</sup>	0,45 <sup>b</sup>	0,47 <sup>b</sup>	0,48 <sup>b</sup>
200E	0,35 <sup>b</sup>	0,38 <sup>b</sup>	0,35 <sup>b</sup>	0,37 <sup>b</sup>	0,39 <sup>b</sup>	0,38 <sup>b</sup>
P<	0,01	0,001	0,001	0,001	0,001	0,001

Figure 1. Evolution of TBARS values.

