

## OXIDATION IN FRESH AND SPRAY-DRIED $\omega$ 3 AND $\omega$ 6 FATTY ACID ENRICHED EGGS VITAMIN E AND CANTHAXANTIN.

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### Summary

A trial with 192 laying hens was conducted to study the effect of Vitamin E and Canthaxanthin as antioxidants in  $\omega$ 3 either  $\omega$ 6 fatty acid (FA) enriched eggs. The animals were randomly assigned to eight treatments resulted from the supplementation of a basal diet containing 5% of linseed oil (L) or sunflower oil (S) with 200 ppm of  $\alpha$ -tocopheryl acetate (LE and SE), 5ppm of Canthaxanthin (LC and SC) or both (LEC and SEC). After 40 days of experimental treatment, eggs were collected and oxidation was measured by the TBA methodology (Botsoglou et al., 1994) on fresh and spray-dried eggs. On fresh eggs there were no differences in TBARS values (expressed as ng MDA/g egg) for any of the studied factors. On spray-dried eggs, the values obtained were 6 to 8-fold higher than in fresh eggs. Eggs from the diets with linseed oil (26,6 % PUFA and 10,4 %  $\omega$ 3) showed higher values than those from diets with sunflower oil (28,0 % PUFA and 26,7 %  $\omega$ 6) (L: 338,7 vs. S: 248,6;  $P < 0,05$ ). The supplementation with Vitamin E resulted in lower TBARS values (E -: 332,9 vs. E +: 254,4;  $p < 0,05$ ) but the supplementation with 5 ppm of Canthaxanthin showed no effect on TBARS reduction (C -: 301,0 vs. C +: 286,3;  $p > 0,05$ ). It can be concluded that  $\omega$ 3 FA enriched eggs are more susceptible to oxidation than  $\omega$ 6 FA enriched eggs. Supplementation with 200 ppm of Vitamin E can reduce the oxidation induced by the spray-drying process, but supplementation with 5 ppm of Canthaxanthin had no significant effect as antioxidant.

### Introduction

Consumption of products enriched in  $\omega$ 3 fatty acids such broiler meat or eggs are associated to health benefits. However, the increase in unsaturation of the lipids present in the food may cause a reduction in its oxidative stability, decreasing its nutritive and organoleptic value and producing of toxic lipid oxidation products.

Supplementation of animal diets with natural antioxidants can prevent the formation of those undesirable compounds. One of the most well known natural antioxidants is vitamin E. Its antioxidant power has been demonstrated in broiler meat (Ajuyah et al, 1993a) and egg products (Wahle et al, 1993).

Much research is being performed in order to find natural antioxidants alternatives to  $\alpha$ -tocopherol. Carotenoids are one of these groups of compounds with antioxidant activity. Antioxidant activity of the red xanthophyll canthaxanthin is controversial. Some authors have reported the *in vitro* antioxidant activity of canthaxanthin (Palozza and Krinsky, 1992; Jorgensen et al, 1993) but its *in vivo* antioxidant activity has been only tested in broilers without clear results (Barroeta and King, 1991; Woodall et al., 1996).

The objective of the present study was to compare the antioxidant activity of canthaxanthin vs. of vitamin E in  $\omega$ 3 and  $\omega$ 6 FA enriched eggs.

### Materials and methods

192 Lohman laying hens were randomly distributed into 8 dietary treatments (24 hens/treatment; 4 hens/replicate). Diets were formulated to meet NRC (1994) requirements. Experimental treatments resulted from the supplementation of a basal diet containing 5% Linseed oil (L) (66,60 % PUFA, 35,55%  $\omega$ 3) or Sunflower oil (S) (61,0 % PUFA; 57,02 %  $\omega$ 6) with 200 ppm of alpha-tocopheryl acetate (LE and SE), 5 ppm of canthaxanthin (LC and SC) or both (LEC and SEC). Oils were obtained from a local supplier and  $\alpha$ -tocopheryl acetate and canthaxanthin were supplied by Roche Vitaminas, Madrid, Spain and Hoffman-La Roche, Base, Switzerland.

After 40 days of feeding, all eggs produced were collected and identified for each replicate 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 fatty acid profile and TBA. The rest of eggs were also cracked, homogenised and frozen at -20 °C until their spray-drying process which was performed at the Escola Superior d'Enginyers Agrònoms de Girona (UdG) by means of a LabPlant Spray-drier SD-05". Conditions of spray-drying were as follows: inlet Temp: 160 °C; outlet Temp: 90°C; air flow: 63 cm<sup>3</sup>/min; air press: 2 bar; egg flow: 0,9L/h.

Oxidation was measured in 6 samples (pool of 5 eggs) per treatment, 3 days after collection by the TBA methodology using the third derivative method as described by Botsoglou et al (1994). Fatty acid profile was determined from feed (3 samples/treatment) and eggs (6 samples/treatment). The total fat of diets and eggs was extracted according to Folch *et al.* (1957) and methylated with 5% boron trifluoride methanol complex in methanolic solution (Morrison and Smith, 1964). The FA profile was determined as described by López-Ferrer et al (1999).

All data were analysed by ANOVA using the SAS ® General Linear Models procedures (SAS Institute, 1996) testing for main (oil, vitamin E and canthaxanthin) and interaction effects.

### Results and discussion

The FA composition of total egg lipids reflected those of the layer diets (table 1). Eggs from hens fed diets with linseed oil showed higher levels of  $\omega$ 3 PUFA than those from hens fed diets with sunflower oil.

TBA values, expressed as ng MDA/g egg are shown in table 2. In fresh egg there were no differences ( $p>0.05$ ) for any of the variation factors. Fresh egg is very stable to lipid oxidation as reported by Pike and Peng (1985). Our results are consistent with those of Marshall et al (1994) and Cherian and col (1996) who shown that  $\omega$ 3 fatty acid enriched eggs held at 4 °C do not show an increase in TBA values within 5-6 weeks. This stability to oxidation can be attributed to the presence of natural antioxidants like tocopherols, carotenoids, metal chelators (Ternes et al, 1997), or by the structure of lipid granules in egg yolk (Pike and Peng, 1985).

When eggs were spray-dried, there was an increase 7-8 fold in the TBA values. Processing of eggs under high temperatures like spray-drying process, partially destroys defensive systems against oxidation, making lipids to be more susceptible to oxidation. Eggs from linseed diets showed values statistically significant higher than those from sunflower diets (338.7 vs. 248.6). This may be due to the higher number of double bonds in the long chain PUFA in L eggs.

Parallel, eggs from diets without vitamin E supplementation showed values 23.6 % higher than those from vitamin E added diets (332.9 vs. 254.4). Supplementation with 200 ppm of vitamin E is an effective method to prevent egg lipid oxidation. Our results confirm the antioxidant effect of vitamin E in eggs previously reported (Wahle et al, 1993).

On the other hand, supplementation with 5 ppm of canthaxanthin had no effect on lipid oxidation. TBA values do not differ between treatments with or without canthaxanthin, either in fresh eggs nor spray-dried eggs. In the bibliography there are no works dealing with the use of canthaxanthin as antioxidant in eggs, and all *in vivo* studies are performed in broilers. Mayne and Parker (1989), Barroeta and King (1991) and Ajuyah et al (1993b) found some protective effect with 5, 3,6 and 10 ppm of canthaxanthin respectively supplemented to broiler diets. On the other hand, Woodall et al (1996) with 100 ppm and Bertelsen et al (1998) with 2 ppm of canthaxanthin did not find any antioxidant effect of this xanthophyll fed to broilers. This lack of effect of canthaxanthin may be due to dosage, kinetics and metabolism, or to its action mechanism. Maybe 5 ppm is not enough to show its antioxidant power but if more than 5-6 ppm are used eggs would not be acceptable from a commercial point of view. Canthaxanthin has been deposited in egg yolk, because yolk coloration (data not shown) reached 13 in the Roche scale, but we do not know how much has been deposited. In the literature its deposition

values differ from 13 % to 38 % (Karunajeewa et al, 1984). Canthaxanthin and astaxanthin are low reactive with free radicals (Mortensen et al, 1997), and this may be the reason of its lack of action. Carotenoids are more efficient than  $\alpha$ -tocopherol at physical quenching of singlet oxygen (Di Mascio et al, 1989). Thus, maybe if the initial factor of oxidation has been singlet oxygen, canthaxanthin could have shown some antioxidant effect.

It can be concluded that  $\omega$ 3 FA enriched eggs are more susceptible to oxidation than  $\omega$ 6 FA enriched eggs. Supplementation with 200 ppm of Vitamin E can certainly reduce the oxidation induced by the spray-drying process, but supplementation with 5 ppm of Canthaxanthin had no significant effect as antioxidant.

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**Table 1. Fatty acid composition of eggs**

%	SAT	MUFA	PUFA	Trans	$\omega$ -3	$\omega$ -6
Linseed oil	30,1	42,7	26,6	0,7	10,4	16,2
Sunflower oil	32,3	39,0	28,0	0,7	1,4	26,7
p<	0,001	0,001	0,005	NS	0,001	0,001

**Table 2. TBA values (ng MDA/g egg) in fresh and spray-dried eggs.**

	Fresh egg	Spray-dried egg
<b>Oil</b>		
Linseed	35,0	338,7
Sunflower	40,1	248,6
p<	NS	0,05
<b>Vitamin E</b>		
0 ppm	34,9	332,9
200 ppm	40,1	254,4
p<	NS	0,05
<b>Canthaxanthin</b>		
0 ppm	36,9	301,0
5 ppm	38,1	286,3
p<	NS	NS

**COMPOSITION AND TASTE OF EGGS ENRICHED WITH OMEGA-3 FATTY ACIDS AND NATURAL ASTAXANTHIN.**

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**Summary**

Laying hen diets, each containing four percent of one of three different fat sources (vegetable fatty acid blend, linseed oil, and fish oil) were supplemented with crushed and spray-dried aplanospores of the green micro alga *Haematococcus pluvialis* to provide 0, 2 or 4 mg of astaxanthin per kg feed. The algal meal was added either as dry powder or mixed with the whole or parts of the portion of fat. The diets were fed to commercial white leghorn laying hens for a 6 week period. Yolk pigmentation and the content of carotenoids, fat, fatty acids, and peroxide and anisidine values in the feeds and eggs were estimated. Fresh and stored eggs were evaluated sensorically.

Pigmentation and the concentration of carotenoids of the yolk were considerably influenced by the dietary concentration of the algal meal (astaxanthin).

Deposition rate of carotenoids and astaxanthin in the egg yolk were 14 and 10%, respectively. Peroxide values of the feed containing linseed oil without added algal meal tended to be elevated, whereas addition of algal meal to these diets decreased the peroxide value. The anisidine value of egg yolks followed the same pattern indicating antioxidant activity of the algal meal.

The content of  $\omega$ -3 fatty acids was highest in eggs from hens fed linseed oil. The content of fatty acids 20:5 plus 22:6 was calculated to 223, 106 and 85 mg per egg, respectively in eggs from hens fed fish oil, linseed oil and vegetable fatty acid blend, respectively.

Consumption of an egg based on vegetable fatty acids would provide approximately 5 per cent and an  $\omega$  -3 enriched egg (linseed oil) approximately 20 per cent of the recommended daily intake of  $\omega$  -3 fatty acids.

Stored eggs based on fish oil were categorized as having a "stronger egg taste" by a taste panel. Otherwise there were no remarkable gastronomic differences, neither in fresh nor in stored eggs, due to fat source or astaxanthin supplementation.