

# EVOLUTION OF $\alpha$ -TOCOPHEROL DEPOSITION IN EGGS ENRICHED WITH POLYUNSATURATED FATTY ACIDS.

Galobart, Jaume, Barroeta, A. C.<sup>1</sup>, Cortinas, L. and Baucells, M.D.

Departament de Ciència Animal i dels Aliments, Facultat Veterinària, Universitat Autònoma de Barcelona. E-08193 Bellaterra, Spain. <sup>1</sup>Ana.Barroeta@uab.es

## Summary

The objective of the present study was to evaluate the evolution of  $\alpha$ -tocopherol concentration in egg during time as a response of dietary supplementation with 200 mg/kg diet of  $\alpha$ -tocopheryl acetate, and the effect of dietary oil on this parameter. Forty hens were randomly distributed into 2 treatments resulting of the supplementation of a basal diet containing 200 mg/kg  $\alpha$ -tocopheryl acetate with 5% linseed oil or sunflower oil.  $\alpha$ -Tocopherol concentration of eggs was measured at 0, 4, 9, 14, 19, and 50 days after inclusion of the experimental diets.  $\alpha$ -Tocopherol concentration in egg increased after the fourth day of feeding, reaching its maximum level at 14 days (168  $\mu$ g/g egg), after which it descended 10-12% until 19 days, remaining constant until 50 days (145  $\mu$ g/g egg). No differences were observed between dietary oils in the deposition pattern of  $\alpha$ -tocopherol.

**Keywords.**  $\alpha$ -tocopherol evolution, PUFA-enriched eggs, dietary supplementation

## Introduction

Because of the health benefits associated with  $\omega$ 3-fatty acids (FA), much research in recent years has focused on enrichment of different foods of animal origin, especially broiler meat and eggs (Ajuyah et al., 1993; López-Ferrer et al., 1999; Baucells et al., 2000). The higher polyunsaturated FA (PUFA) content of these foods leads to the increase in their unsaturation and, thus, to a major susceptibility to lipid oxidation. In order to improve oxidative stability of such products, supplementation with tocopherols to hen's diet has been successfully used (Cherian et al., 1996; Qi and Sim, 1998; Galobart et al., 2001).

Although it has been stated that  $\alpha$ -tocopherol ( $\alpha$ -Toc) content in eggs depends on its concentration in the diet (Frigg et al., 1992; Jiang et al., 1994; Meluzzi et al., 1999) several factors could affect its deposition, such as the amount and type of fat added to the diet (Frigg et al., 1992; Grobas, 1997; Meluzzi et al., 2000). However, very few studies dealt with the evolution of  $\alpha$ -Toc deposition in eggs during time as a result of dietary supplementation with  $\alpha$ -tocopheryl acetate ( $\alpha$ -TA) (Surai et al., 1995; Chen et al., 1998; Meluzzi et al., 1999), and to our knowledge, there are no works studying the effect of dietary fat on this parameter. The objective of the present study was to evaluate  $\alpha$ -Toc deposition in eggs enriched with  $\omega$ 3 or  $\omega$ 6 PUFA during supplementation time.

## Materials and methods

Forty Lohmann hens were randomly distributed into two experimental treatments (5 replicates of 4 hens per treatment). Diets were formulated to meet or exceed NRC (1994) requirements and resulted from the supplementation of a basal diet containing 5% linseed oil (LO) or sunflower oil (SO) with 200 mg/kg  $\alpha$ -TA. At 0, 4, 9, 14, 19 and 50 d after the inclusion of the experimental diets, four eggs were collected from each replicate. These eggs were then cracked, homogenized and frozen at  $-20^{\circ}\text{C}$  until its analysis for  $\alpha$ -Toc content.

$\alpha$ -Toc from feed was analysed following Manz and Philipp (1981).  $\alpha$ -Toc from fresh eggs was extracted using the method described by Abdollahi et al. (1993) and HPLC determination was performed using the conditions described by Drotleff and Ternes (1999).

ANOVA was performed to determine whether the type of fat and supplementation time affected  $\alpha$ -Toc content in egg.

## Results and discussion

$\alpha$ -Toc content of the diets were 224.6 and 242.9 mg/kg of feed for LO and SO treatments respectively.

Table 1 shows the results obtained in the evolution of  $\alpha$ -Toc content in eggs during the 50 d of the feeding trial. After 4 d of supplementation, there is a fast increase in  $\alpha$ -Toc content of egg, reaching the maximum value at 14 d. After this time, there  $\alpha$ -Toc content is reduced by 10-12% until 19 d, after which,  $\alpha$ -Toc concentration remains almost constant until 50 d. No differences were observed between dietary oils on this pattern of deposition of  $\alpha$ -Toc in eggs.

**Table 1.** Evolution of  $\alpha$ -tocopherol deposition ( $\mu$ g/g egg) in eggs depending on supplementation time and dietary fat.

Time (days)	Linseed oil	Sunflower oil	SEM	P
0	28,8 <sup>c</sup>	29,3 <sup>d</sup>	0,94	NS
4	45,9 <sup>d</sup>	45,7 <sup>c</sup>	2,65	NS
9	130,5 <sup>c</sup>	156,5 <sup>b</sup>	4,64	**
14	164,4 <sup>a</sup>	172,3 <sup>a</sup>	4,15	NS
19	144,0 <sup>b</sup>	154,8 <sup>b</sup>	5,78	NS
50	147,1 <sup>b</sup>	142,7 <sup>b</sup>	7,23	NS
SEM	4,07	5,62		
P	***	***		

NS = Not significant; \*\* =  $P \leq 0,01$ ; \*\*\* =  $P \leq 0,001$ . SEM = Standard Error of the Means <sup>a,b,c,d</sup> Different superscripts for a certain oil indicate significant differences between supplementation time.

Our results indicate that  $\alpha$ -Toc contents of eggs is easily modifiable through dietary supplementation, and are in general in concordance with those published by other authors, with some differences.

Surai et al. (1995) observed that  $\alpha$ -Toc deposition in eggs from hens fed diets with 200, 2000, and 20000 mg  $\alpha$ -Toc/kg of diet, increased in a dose dependent manner until 18-20 d. After this time,  $\alpha$ -Toc concentration remained constant for two weeks and then declined during the following 4 weeks by 50%, and maintaining this level if dietary supplementation was kept. Meluzzi et al., (1999) observed a similar evolution. Feeding hens with diets containing 200 mg/kg  $\alpha$ -TA, observed that  $\alpha$ -Toc content of eggs doubled every week, reaching its maximum (330  $\mu$ g/g egg yolk) at 3 weeks.  $\alpha$ -Toc content was reduced during the fourth week

at 210 µg/g egg yolk, remaining constant during 3 weeks. Chen et al. (1998) reported that α-Toc content of eggs increased in a dose-dependent manner after the third day of feeding, reaching its maximum at 14 days, after which it remained constant until the end of the trial (28 d). Although the same tendency was observed in the deposition pattern, the values reached when the α-Toc concentration stabilizes are quite different. So, while in our study, α-Toc concentration at 19 d was 150 µg/g egg (approx. 450 µg/g egg yolk), Meluzzi et al. (1999) and Surai et al. (1995) with similar levels of supplementation (200 mg/kg α-TA), obtained 210 and 1200 µg/g egg yolk respectively, and Chen et al. (1998) with 120 mg/kg diet obtained 70 µg/g egg yolk. Moreover, differences were observed between studies in the proportion of reduction in the α-Toc content after reaching its maximum level. So, while in our case α-Toc content was reduced by 10-12%, Meluzzi et al. (1999) and Surai et al. (1995) observed a higher reduction (30% and 47-57% respectively). On the other hand, Chen et al. (1998) did not observe any reduction during the 28 d trial. These differences observed in the reduction as well as in the stabilized concentration may be attributed to several factors such as the strain of hen, laying parameters, the different experimental conditions and analytical procedures used, as well as the kind and amount of α-Toc used. But probably the more important factor which causes the main differences between works are those related to the hen's diet, like differences in the α-Toc content of the basal diets, the presence of other antioxidants (e.g., ethoxyquin, BHT) that could act sparing α-Toc, and thus, increasing the amount of this compound available to be deposited into eggs. Other feed ingredients, such vitamin A (Grobas, 1997) and the type and amount of fat, could affect α-Toc transfer to the egg. Regarding the type of fat, no works were found studying the effect of the dietary fat on the α-Toc deposition pattern to egg. Some authors have found that α-Toc concentration in eggs was higher in those treatments with a lower level of fat unsaturation (Frigg et al., 1992; Grobas, 1997; Meluzzi et al., 2000). Although in our case, no differences were found between dietary oils, except at 9 d, in a previous work (Galobart et al., 2001) we observed that α-Toc concentration was higher in eggs from treatments with 5% SO, with a lower level of unsaturation (66.1% unsaturated fatty acids) than in treatments with 5% LO (69.3% unsaturated fatty acids). Our observations and those from other authors seems to support the theory that α-Toc deposition to eggs diminishes when dietary unsaturation level increases. Some authors postulate that high levels of PUFA in the diet reduce α-Toc absorption in the gastrointestinal tract (Hollander, 1981), maybe affecting lipid micelle properties, and α-Toc partition between micelles and intestinal mucose (Combs, 1996; Hollander, 1981). On the other hand, the higher ω3 PUFA content in eggs from LO treatments leads to a higher oxidative susceptibility in the animal tissues, which contributes to a major α-Toc consume, and thus, less antioxidant is free to be deposited into egg.

In conclusion, a minimum of 3 weeks are needed to achieve an stabilized α-Toc content in the egg. The type of dietary fat may affect α-Toc content of eggs but do not affect its deposition pattern.

#### Acknowledgements

This work was supported in part by a grant from *Comissió Interdepartamental de Recerca i Innovació Tecnològica* (CIRIT), and *Comisión Interministerial de Ciencia y Tecnología* (CICYT).

#### References

- Abdollahi, A., Rosenholtz, N.S. & Garwin, J.L. (1993) Tocopherol micro-extraction method with application to quantitative analysis of lipophilic nutrients. *Journal of Food Science* 58: 663-666.
- Chen, J.Y., Latshaw, J.D., Lee, H.O. & Min, D.B. (1998) α-tocopherol content and oxidative stability of egg yolk as related to dietary α-tocopherol. *Journal of Food Science* 63: 919-922.
- Cherian, G., Wolfe, F.W. & Sim, J.S. (1996) Dietary oils with added tocopherols: Effects on egg or tissue tocopherols, fatty acids and oxidative stability. *Poultry Science* 75: 423-431.
- Combs, G.F. (1996) Mechanisms of absorption, transport and tissue uptake of vitamin E, in: Coelho, M.B. (Ed) *Vitamin E in animal nutrition and management*, pp 27-36 (BASF Corporation).
- Drotleff, A.M. & Temes, W. (1999) Cis/trans isomers of tocotrienols-occurrence and bioavailability. *European Food Research and Technology* 210: 1-8.
- Frigg, M., Whitehead, C.C. & Weber, S. (1992) Absence of effects of dietary α-tocopherol on egg yolk pigmentation. *British Poultry Science* 33: 347-353.
- Galobart, J., Barroeta, A.C., Baucells, M.D. & Guardiola, F. (2001) Lipid oxidation in fresh and spray-dried eggs enriched with ω3 and ω6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and canthaxanthin supplementation. *Poultry Science* 80: 327-337.
- Grobas, S. (1997) Influencia de la nutrición sobre el tamaño y la calidad interna del huevo. *Ph. D. Thesis*, Universidad Politécnica de Madrid.
- Hollander, D. (1981) Intestinal absorption of vitamins A, E, D and K. *Journal of Laboratorial and Clinical Medicine* 97: 449-462.
- Jiang, Y.H., McGeachin, R.B. & Bailey, C.A. (1994) α-tocopherol, β-carotene, and retinol enrichment of chicken eggs. *Poultry Science* 73: 1137-1143.
- Manz, U. & Philipp, K. (1981) A method for the routine determination of tocopherols in animal feed and human foodstuffs with the aid of high performance liquid chromatography. *International Journal Vitamin Nutrition Research* 51: 342-348.
- Meluzzi, A., Sirri, F., Tallarico, N. & Vandì, L. (1999) Dietary vitamin E in producing eggs enriched with n-3 fatty acids. *Proceedings of the VIII European Symposium on the Quality of Eggs and Egg Products*, Bologna, pp. 153-159.
- Meluzzi, A., Sirri, F., Manfreda, G., Tallarico, N. & Franchini, A. (2000) Effects of dietary vitamin E on the quality of table eggs enriched with n-3 long-chain fatty acids. *Poultry Science* 79: 539-545.
- Qi, G.-H. & Sim, J.S. (1998) Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. *Journal of Agricultural and Food Chemistry* 46: 1920-1926.
- Surai, P.F., Ionov, I.A., Buzhin, A. & Buzhina, N. (1995) Vitamin E and egg quality. *Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products*, Zaragoza, pp. 387-394.