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Short communication. Effects of type and level of supplementation with dietary n-3 fatty acids on yolk fat composition and n-3 fatty acid retention in hen eggs

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Abstract

A marine algal oil (MAO) with a high docosahexaenoic acid content (46.7%) was evaluated for the production of n-3 fatty acid (FA) enriched eggs. To determine its effects, laying hens were fed one of two diets containing different amounts of MAO, or a control diet containing marine fish oil (MFO). The efficiency of retention of total n-3 FA in the yolk fat depended both on the type and level of dietary n-3 substrate. Per unit weight of dietary oil added, MAO was better than MFO in terms of total n-3 FA retention by the yolk fat. However, the efficiency of retention of total n-3 FA, expressed as g FA retained per g of FA intake, was higher for MFO than for MAO.

Additional key words: layers, n-3 retention efficiency, n-3 sources, yolk fatty acid composition.

Resumen

Efecto del tipo y nivel de suplementación con ácidos grasos n-3 en la ración sobre la composición de la grasa de la yema y la retención de ácidos grasos n-3 en huevos de gallinas ponedoras

Un aceite procedente de algas marinas (AM) y con una alta concentración de ácido docosahexaenoico (46,7%) fue evaluado para producir huevos enriquecidos en ácidos grasos (AG) n-3. Se formularon tres dietas experimentales para comparar esta nueva fuente a dos niveles de inclusión con una dieta control suplementada con aceite de pescado (AP) en gallinas ponedoras. Los resultados indican que la eficacia de retención de AG n-3 en la grasa de la yema depende tanto del tipo como del nivel de suplementación de la fuente de AG n-3 utilizada. La suplementación con AM fue más eficiente que la de AP sobre la base del total de AG n-3 retenidos en la grasa de la yema por unidad de peso de aceite añadido. Sin embargo, la eficacia de deposición del total de AG n-3, expresada como gramos de AG retenidos por gramo de AG consumidos, fue mayor para AP que para AM.

Palabras clave adicionales: composición en ácidos grasos de la yema, eficacia de retención de n-3, fuentes de n-3, ponedoras.

The production of n-3 enriched eggs has increased in Spain over recent years, and presently accounts for some 3% of the total layer-egg market. Marine fish oil (MFO) is the preferred source of dietary long-chain n-3 fatty acids (LCn-3 FA) on the basis of its cost and efficiency of retention in yolk fat. Recently, a vegetable source, marine algal oil (MAO), has become available on the Spanish market. MAO is more expensive than MFO, but its docosahexaenoic acid (DHA) content is about double (46.8 compared to 22.0%), while its concentration of eicosapentaenoic acid (EPA) is similar (8.4 and 8.0% respectively).

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Several papers, reviewed by González-Esquerra and Leeson (2001), indicate the high efficiency of DHA retention in eggs (about 50%). Accordingly, the efficiency of retention of total LCn-3 FA might be greater with MAO than MFO (Herber-McNeill and Van Elswyk, 1996). Further, high doses of MFO have been associated with a reduction in egg sensorial quality due to the presence of off-flavours (Noble, 2001). MAO, however, maintains consumer acceptability (Herber-McNeill and Van Elswyk, 1998).

The efficiency of LCn-3 FA retention also depends on the level of dietary supplementation. Previous work with MFO (Adams *et al.,* 1989; Huang *et al.,* 1990) and MAO (Herber-McNeill and Van Elswyk, 1996; Abril *et al.,* 2000) has shown the yolk retention rate

for LCn-3 FA to be reduced with increasing dietary concentration. However, González-Esquerra and Leeson (2000) report a linear response of yolk LCn-3 FA concentration for up to a 6% level of inclusion of menhaden oil.

The aim of the present study was to compare the yolk fat retention of LCn-3 FA [with a high DHA content (46.8%)] provided by MAO, at two levels of dietary inclusion. The results were compared to a control diet that included a commercial source of MFO.

The experimental birds were thirty six 58 week-old Warren laying hens housed individually in cages (33 cm × 41 cm). The trial lasted for 42 d, following a pre-experimental period of 14 d. Feed was restricted to 115 g d^{-1} while water was supplied *ad libitum*. No feed refusals were observed. The hens were provided with 15 h of light per day throughout the experiment. The temperature was maintained at about 21ºC.

The hens were randomly assigned to one of three dietary treatments, T1, T2 or T3. These diets were formulated by substituting the corresponding part of the base diet with 1.7% MFO, 0.77% MAO or 1.7% MAO. The concentrations of DHA and EPA were 22% and 8% respectively in the MFO, and 46.8% and 8.4% in the MAO. The proportions of C16:0, C18:0, C18:1, C18:2 and C18:3 were 18.3, 5.3, 16.0, 1.2 and 0.5%, and 2.6, 2.8, 6.8, 0.6 and 0.2% in the MFO and MAO respectively. Accordingly, diets T1 and T2 had a similar DHA content (0.36%) but differed in their EPA content (0.14% compared to 0.06% respectively). The EPA contents of diets T1 and T3 were similar (0.14%), but the DHA content of diet T3 (0.80%) was greater than that of diet T1 (0.36%). Table 1 shows the ingredients and chemical compositions of the base diet.

Hen-day egg production and egg weight per hen were recorded daily throughout the trial. The body weights of the birds were recorded at the beginning and end of the experimental period. All eggs produced during the third and last week of the experimental period were kept at 4ºC for less than seven days to determine the yolk weight and to analyse the yolk FA composition. The average values for each hen over these two weeks (twelve hens per treatment) were used in statistical analyses. The yolk lipids of three eggs (selected at random and pooled) per hen were extracted following the method of Folch *et al.* (1957). The fatty acid profiles (two analyses per replicate) of experimental fats and egg yolks were determined according to Cherian and Sim (1992). The fat extracted from each sample was methylated (Metcalfe *et al.,* 1961), and the FAs separated and identified using a Hewlett-Packard 5890 gas chromatograph (Varian; Walnut Creek, California, USA) equipped with a Supelco SP-2330 (30 m × 0.25 mm inside diameter) silica capillary column. The apparatus was programmed with an initial temperature of 170ºC for 20 min, allowing increases of 5.4ºC min^{-1} until a final temperature of 250ºC was reached. The temperature of the injector and detector was 250ºC. Hydrogen at 11.5 psi was used as the carrier gas. The calibration and identification of the peaks for the different FAs was performed by comparing retention times with that of a known standard (Qualimix Fich S. Ref. 89-5550 larodan; Malmoe, Sweden).

The efficiency of polyunsaturated n-3 FA retention was calculated as the ratio between the FA retained in the yolk fat (estimated as laying rate × yolk weight × yolk fat content × proportion of FA in the yolk fat) and FA ingested (estimated as feed intake x dietary fat

Table 1. Composition (g kg^{-1}) and nutrient analysis of the base diet

Ingredients	
Wheat	317.5
Yellow corn	303.5
Full fat soybean	70.0
Soybean meal (44% crude protein)	164.4
Corn dry distillers grains and solubles	20.2
Dicalcium phosphate	8.30
Calcium carbonate	104.2
Sodium chloride	2.83
Sodium bicarbonate	1.68
Alimet®[a]	1.31
Vitamin-mineral premix[b]	3.40
Formic acid	2.50
Calculated variables[c]	
ME, kcal kg^{-1}	2,600
Crude protein, %	15.8
Lysine, %	0.78
Methionine, %	0.37
TSAA, %	0.66
Ether extract, %	3.46
Calcium, %	4.28
Total P, %	0.51

[a] 2-hydroxy 4-methyl thiobutanoic acid (Novus International St. Louis, Missouri). [b] Supplying (mg kg^{-1} of diet): retinyl acetate, 3.4; cholecalciferol, 0.075; DL-α-tocopherol, 13; menadione, 2; riboflavin, 4; panthotenic acid, 8; nicotinic acid, 30; pyridoxine, 0.8; folic acid, 0.25; D-biotin, 0.05; thiamine, 1; cyanocobalamin, 0.01; choline chloride, 250; Mn, 80; Zn, 65; Fe, 50; Cu, 8; I, 1.9; Se, 0.25; canthaxantin, 3; lutein, 6; ethoxyquin, 125. [c] According to FEDNA (2003).

content × FA concentration in dietary fat). An internal standard (trimargarolein; Sigma-Aldrich Corporation, St. Louis, Missouri, USA) was used to measure the concentration of total FA in the yolk and dietary fat.

The effects of inclusion of n-3 sources in the base diet were analysed (considering the experiment to have a completely randomised design) using SAS software (SAS Institute Inc., 1990). Non-orthogonal contrasts were used to test the effects of the different dietary levels of EPA (T1 *vs.* T2) and DHA (T1 *vs.* T3).

The type of diet affected neither weight gain, laying rate, egg weight nor yolk weight, whose means were –4.16 g (± 27.0, SE), 81.9% (± 2.47), 67.6 g (± 1.77) and 18.1 g (± 0.53) respectively. The treatments did not affect total yolk fat content (mean 32.7% ± 0.11), but did influence the FA profile of the egg yolk (Table 2). An increase in dietary EPA concentration from 0.06% to 0.14% had no effect ($P > 0.10$) on the yolk fat content of saturated (SAT), monounsaturated (MUFA) or n-6 polyunsaturated FA (PUFA), but increased those of EPA, $C_{22:5\ n-3}$ (DPA), DHA and total n-3 FA.

Further, the efficiency of retention (expressed as g FA retained/100 g FA ingested) of EPA was lower ($P < 0.001$) and that of DHA higher ($P < 0.001$) in treatment T1 than in T2. No effect of EPA addition was therefore found on the efficiency of retention of total n-3 FA. An increase in the dietary DHA concentration from 0.36% (treatment T1) to 0.80% (treatment T3) decreased the total yolk fat MUFA and $C_{20:4\ n-6}$ contents by 4.3% and 15.7% respectively, but increased those of SAT, linolenic, EPA, DPA, DHA and total n-3 FA. The efficiency of retention of DHA and total n-3 FA was poorer ($P < 0.001$) in treatment T3 than in T1, whereas only a small difference ($P = 0.1$) was found for EPA retention efficiency. As a consequence of the previous effects, total n-3 FA concentration per egg

Table 2. Effect of treatments on yolk fatty acid composition, n-3 fatty acid retention efficiency, and egg total n-3 concentration

	Treatment[a]			SEM[b]	Significance	
	T1	T2	T3		T1 *vs* T2	T1 *vs* T3
Fatty acid						
Total SAT	33.5	33.1	34.8	0.29	0.34	0.003
C16:0	24.4	24.3	25.5	0.28	0.76	0.01
C18:0	8.41	8.25	8.77	0.17	0.51	0.14
Total MUFA	49.2	49.5	47.1	0.44	0.61	0.002
C16:1 n-7	3.21	3.32	3.00	0.08	0.39	0.08
C18:1 n-9	45.2	45.6	43.4	0.44	0.59	0.006
Total PUFA n-6	13.0	13.6	13.2	0.28	0.15	0.59
C18:2	11.8	12.3	12.2	0.28	0.19	0.36
C20:4	1.08	1.15	0.91	0.033	0.12	0.001
Total PUFA n-3	3.80	3.35	4.47	0.12	0.01	0.001
C18:3	0.355	0.371	0.392	0.013	0.4	0.05
EPA	0.135	0.110	0.197	0.007	0.021	0.001
DPA	0.165	0.125	0.197	0.009	0.003	0.020
DHA	3.15	2.75	3.69	0.11	0.01	0.002
Total PUFA n-3 concentration (mg $100g^{-1}$ egg)	221	191	250	9.88	0.04	0.04
Efficiency of PUFA n-3 retention in yolk fat[c]						
EPA	4.51	6.64	5.47	0.41	0.001	0.1
DHA	38.2	30.6	18.7	1.12	0.001	0.001
Total PUFA n-3	28.2	29.0	17.8	0.91	0.58	0.001

[a] Treatments represent the type and level of fat added to the base diet: T1 = 1.70% MFO; T2 = 0.77% MAO, and T3 = 1.70% MAO. [b] n=12 per treatment. [c] Determined as total g PUFA n-3 retained in eggs/total g PUFA n-3 ingested (%).

increased as a result of both EPA (from 191 mg in treatment T2 to 221 mg in treatment T1) and DHA addition (up to 250 mg in treatment T3).

Given that the total n-3 FA yolk fat concentration was 13.1% higher for MAO than for MFO at the same level of dietary inclusion, the present results confirm those of other authors (González-Esquerra and Leeson, 2001) indicating DHA to be more efficiently retained in the yolk fat than EPA. However, DHA retention efficiency decreased greatly from 30.6% to 18.7% when the dietary MAO content increased from 0.77% to 1.70%. These values are lower than the mean efficiencies reported by González-Esquerra and Leeson (2001) at lower mean levels of DHA supplementation, but close to those obtained by Abril *et al.* (2000) at similar levels of DHA intake.

Table 2 shows that when dietary DHA content was increased by more than double (122%) but at a similar level of EPA (treatment T3 *vs.* treatment T1), the yolk DHA concentration only increased by 17%, but those of its metabolic substrates —linolenic, EPA and DPA— increased by 10.4%, 45.9% and 19.4% respectively. This suggests that a high yolk DHA concentration might reduce the activity of enzymes involved in the synthesis of LCn-3 FA. Consequently, the overall efficiency of DHA and total n-3 FA retention decreased sharply (by 51.0% and 36.9% respectively) in hens fed diet T3 compared to those fed diet T1.

The present results also indicate that an increase in dietary EPA at a low DHA concentration (0.37%) implies an increase in the yolk fat content of EPA, but also of DPA and DHA, which are synthesised from EPA. A similar effect has been described by Herber and Van Elswyk (1996). As a consequence, the overall efficiency of retention of EPA was 32.1% lower when increasing the dietary EPA content (treatment T1 *vs.* treatment T2) while that of DHA increased by 24.8%.

The efficiency of retention of FA n-3 in yolk fat appeared to be highly and negatively dependent on the level of dietary DHA supplementation. As a consequence, the total n-3 FA concentration of the egg yolk was higher with MAO than with MFO when equally included in the diet. However, a greater efficiency of retention of DHA and a greater amount of total yolk fat n-3 FA were observed with MFO than with MAO when compared at similar dietary levels of DHA or EPA.

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