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Effect of conjugated linoleic acid, high-oleic sunflower oil and fish oil dietary supplementation on laying hen egg quality

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Abstract

An experiment was performed to determine the effect of supplementing the diet of laying hens with conjugated linoleic acid (CLA), fish oil (FO) and high-oleic sunflower oil (HOSO) on hen performance, egg yolk fatty acid (FA) concentrations, and egg sensorial quality and firmness. Four treatments were factorially designed involving two levels of supplementation of FO (0 and 17 g kg^{-1}) and HOSO (30 and 35 g kg^{-1}) for diets containing 3 g kg^{-1} of CLA. A control diet was also designed, with 30 g kg^{-1} HOSO but with no CLA or FO. Twenty five 38 week-old Warren laying hens were randomly assigned to 28 d dietary treatments with the above diets, following a pre-experimental period of 21 d. The type of diet did not affect egg production characteristics. The addition of CLA increased the yolk fat concentrations of CLA, saturated FA and $C_{22:5\ n-3}$, but reduced those of monounsaturated FA and $C_{20:4\ n-6}$. Supplementation with FO increased long-chain n-3 FA concentrations but reduced those of long-chain n-6 FAs, t_{10},c_{12} and tt-CLA. Supplementation with HOSO had little influence on yolk fat composition. Supplementation with CLA and FO impaired egg sensorial quality in an additive fashion, although yolk firmness was not affected. Eggs from hens fed diets with 3 g CLA kg^{-1} and 30 g HOSO kg^{-1} —but no added FO— contained 9 g CLA kg^{-1} yolk fat and were of acceptable sensorial quality to trained panellists. However, no eggs double-enriched in CLA and n-3 FA were obtained that were also sensorially acceptable.

Additional key words: CLA, egg quality, layers, n-3 fatty acids.

Resumen

Efecto sobre la calidad del huevo en gallinas ponedoras de la suplementación del pienso con ácido linoleico conjugado, aceite de girasol rico en ácido oleico y aceite de pescado

Se realizó un experimento para evaluar el efecto de la inclusión en la dieta de ácido linoleico conjugado (ALC), aceite de pescado (AP) y aceite de girasol rico en oleico (AGRO) sobre la productividad de gallinas ponedoras, la composición de la grasa de la yema y la calidad sensorial y firmeza de los huevos. Se formularon cuatro piensos combinando factorialmente dos niveles de suplementación de AP (0 y 17 g kg^{-1}) y AGRO (30 y 35 g kg^{-1}) en raciones que contenían una cantidad fija (3 g kg^{-1}) de ALC. Se formuló también un pienso control con 30 g AGRO kg^{-1}, pero sin adición de AP ni ALC. Veinticinco gallinas de estirpe Warren de 38 semanas de edad se asignaron al azar a los tratamientos a lo largo de 28 días, tras un periodo de adaptación de 21 d. Los tratamientos no afectaron a los parámetros productivos de las gallinas. La adición de ALC aumentó la concentración de ALC, ácidos grasos (AG) saturados y $C_{22:5\ n-3}$, pero disminuyó los de AG monoinsaturados y $C_{20:4\ n-6}$ en la grasa de la yema. La suplementación con AP aumentó el contenido en AG n-3 de cadena larga, disminuyendo las de n-6, t_{10},c_{12} y tt-ALC, mientras que el incremento de adición de AGRO tuvo poca influencia sobre la composición de la grasa de la yema. Tanto la suplementación con ALC como con AP empeoró de forma aditiva la calidad sensorial de los huevos, pero la firmeza de la yema no fue afectada por los tratamientos. Huevos de gallinas alimentadas con piensos con 3 g ALC kg^{-1} y 30 g AGRO kg^{-1} sin AP añadido, contenían 9 g ALC kg^{-1} de grasa de la yema y tenían una aceptable calidad sensorial para los consumidores. Sin embargo, no se pudieron obtener huevos doblemente enriquecidos en ALC y AG n-3 con una buena aceptación por parte de los panelistas.

Palabras clave adicionales: ácidos grasos n-3, ALC, calidad de huevo, puesta.

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Introduction

Previous work has shown that supplementing the diet of laying hens with conjugated linoleic acid (CLA) increases the yolk fat CLA concentration of their eggs, but also increases the degree of yolk fat saturation. Even supplementation with as little as 5 g kg^{-1} can lead to a 33-46% increase in total yolk saturated fatty acids and reduce the level of total monounsaturated fatty acids to below 300 g kg^{-1} (Aydin *et al*., 2001; Cherian *et al*., 2002; Szymczyck and Pisulewski, 2003; Álvarez *et al*., 2004b). As a consequence, the hardness of the yolk of cooked eggs increases and the sensorial quality (for consumers) is reduced (Ahn *et al*., 1999; Aydin *et al*., 2001, Álvarez *et al*., 2004a,b; 2005). The combined incorporation of high-oleic sunflower oil (HOSO, 30 g kg^{-1}) and moderate levels of CLA (up to 2 g kg^{-1}) can restore oleic acid yolk fat concentration to above 410 g kg^{-1}, and help produce eggs with an appreciable level of CLA (6.82 g kg^{-1} yolk fat) and acceptable sensorial quality (Álvarez *et al*., 2005).

Other studies indicate that CLA supplementation of fish oil- (FO) or flaxseed-added diets does not reduce and can even increase total n-3 fatty acid deposition in the yolk fat (Du *et al*., 2000; Cherian *et al*., 2002; Raes *et al*., 2002; Álvarez *et al*., 2004b). Further, CLA is an effective antioxidant (Ha *et al*., 1990), and might reduce the off-flavours associated with the feeding of fish oil (Herber-McNeill and Van Elswyk, 1998; Leeson *et al*. 1998). However, the addition of FO to the diet does not reduce the firmness of hardboiled egg yolks from hens receiving CLA-supplemented diets (Álvarez *et al.,* 2004b), and has been associated with reduced sensorial quality caused by the presence of off-flavours [for a review see González-Esquerra and Leeson (2001)].

This study determined the effects of incorporating different levels of FO (0 or 17 g kg^{-1}) and high-oleic sunflower oil (30 or 35 g kg^{-1}) (in combination) to diets containing 3 g of CLA kg^{-1} in an attempt to produce eggs enriched in CLA and n-3 fatty acids with an acceptable sensorial quality for consumers.

Material and Methods

Twenty five 38 week-old Warren laying hens were individually housed in cages (33 × 41 cm). Each hen was considered as one experimental unit. The dietary trial lasted for 28 d and started after a pre-experimental period of 21 d, during which the birds were allowed to adapt to the experimental diets. The length of the pre-experimental period was considered sufficient since previous research (Chamruspollert and Sell, 1999) has shown that the maximum effects of dietary CLA on yolk fat occurs 10-11 days after feeding a CLA source. Feed was restricted to 115 g d^{-1}; water was supplied *ad libitum*. All hens received 15 h light d^{-1} over the entire experimental period. Room temperature was controlled at close to 24ºC.

The hens were randomly assigned to receive one of five dietary treatments (five hens per treatment). Four of these were designed by factorially combining two levels of FO (0 and 17 g kg^{-1}) and HOSO (30 and 35 g kg^{-1}) to a base diet containing a fixed supplement of CLA (3 g kg^{-1}). The fifth was a control diet containing 30 g HOSO kg^{-1}, but no CLA or FO. The CLA source used in this study was obtained from BASF Española, S. A. (Tarragona, Spain). This contained 560 g kg^{-1} CLA;

Table 1. Ingredients and chemical composition of the base diet (g kg^{-1} as fed basis)

Ingredients	
Corn grain	662.2
Soybean meal 47%	214.1
Calcium carbonate	104.4
Calcium bicarbonate	1.8
Dicalcium phosphate	8.5
Sodium chloride	2.9
Formic acid	2.0
Alimet	1.8
Lysine, 50%	0.2
Threonine	0.1
Vitamin-mineral premix[1]	2.0
Calculated contents[2]	
Metabolisable energy (MJ kg^{-1})	11.1
Crude protein	155
Lysine	8.0
Methionine	4.0
Crude fibre	26.8
Starch	422
Ether extract	24.8
Calcium	40.6
Available phosphorus	2.9

[1] Compounds supplied (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; ethoxyquin, 125, phytases, 80[3]. [2] According to FEDNA (2003). [3] Natuphos, 5000 FTU g^{-1}, BASF Española, S. A., Tarragona, Spain.

therefore the actual amount of CLA source added was 5.4 g kg^{-1}. The FO used contained 68 g kg^{-1} of eicosapentanoic acid (EPA) and 184 g kg^{-1} of docosahexanoic acid (DHA), whereas the HOSO contained 757 g kg^{-1} of oleic and 144 g kg^{-1} of linoleic acid. Table 1 shows the ingredients and chemical composition of the base diet; Table 2 shows the fatty acid (FA) profiles of the experimental diets.

Yolk and dietary lipids were extracted following the methods of Folch *et al*. (1957) and OJ (1998) respectively. Fatty acid profiles for the experimental fats, diets and the egg yolks obtained 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 Star 3400 CX, Walnut Creek, CA, USA) equipped with a Supelco SP-2330 (30 m × 0.25 mm inside diameter) silica capillary column. The apparatus was programmed to provide an initial temperature of 150ºC for 4 min, with steps of 1.3ºC min^{-1} until a final temperature of 210ºC was reached. The temperature of the injector and detector was 250ºC. Hydrogen, at 11.5 psi, was used as the carrier gas. Calibration was performed and the different FA peaks identified by comparing retention times with that of a standard (Qualimix Fich S. Ref: 89-5550, LARODAN, Malmö, Sweden) of known composition. Yolk and albumen acidity (973.41) and moisture (920.116) were determined according to AOAC methods (2000).

Hen-day egg production and feed consumption were measured daily throughout the trial. Three eggs produced by each hen at the end of the 28 d experimental period were used to determine egg, albumen and yolk weight and yolk fat content, and to analyse the FA composition of the yolk fat. Pooled data were used for statistical analyses. In addition, six eggs per replicate were collected at the end of the experimental period. Three were used to determine their commercial value on the basis of shell thickness, albumen height and yolk colour, as measured by the Roche yolk colour fan (Vuilleumier, 1969), the remaining three were used to measure albumen and yolk pH and moisture.

Twelve additional eggs per treatment were randomly selected for sensorial evaluation. All were kept at 5ºC for 14 d. They were then boiled for 15 min and kept in warm water until tasted (Caston and Leeson, 1990). A panel of judges was selected from among experienced personnel belonging to the COREN Quality Control Department; UNE rules were followed to ensure reproducibility. The selection criteria included the ability to discriminate standard flavours (UNE 87.017.92) and

Table 2. Fatty acid profiles of the ether extracts of the experimental diets (g kg^{-1})

Treatments	T1	T2	T3	T4	T5
CLA supplement, g kg^{-1}	0	3	3	3	3
HOSO supplement, g kg^{-1}	30	30	30	35	35
FO supplement, g kg^{-1}	0	0	17	0	17
$C_{14:0}$	0.08	0.05	0.66	0.05	0.56
$C_{16:0}$	5.25	4.62	7.42	4.70	6.87
$C_{16:1\ n-7}$	0.14	0.09	0.86	0.10	0.75
$C_{18:0}$	1.77	2.00	2.81	2.09	2.58
$C_{18:1\ n-9}$	25.6	29.4	32.5	30.9	33.3
$C_{18:2\ n-6}$	17.4	16.4	17.0	16.0	15.9
$C_{18:3\ n-3}$	0.78	0.68	0.86	0.64	0.82
$C_{20:4\ n-6}$	0.24	0.34	0.37	0.33	0.35
$C_{20:5\ n-3}$	0.00	0.00	0.94	0.00	0.82
$C_{22:6\ n-3}$	0.00	0.00	2.52	0.00	2.18
c_9,t_{11} CLA	0.00	1.60	1.58	1.45	1.84
t_{10},c_{12} CLA	0.00	1.54	1.51	1.39	1.70
Total CLA	0.00	3.14	3.10	2.84	3.54
Total SFA	7.10	6.66	10.9	6.84	10.0
Total MUFA	25.8	29.5	33.4	31.0	31.4
Total non-CLA n-6 FA	17.6	16.7	17.4	16.3	16.3
Total n-3 FA	0.78	0.68	4.32	0.64	3.81

SFA: total saturated fatty acids. MUFA: total monounsaturated fatty acid.

the consistency and reproducibility of scores awarded in accordance with the intensity of sensations perceived (UNE 87.020.93). The results obtained from the preliminary tasting sessions led to twelve panellists being selected. These were asked to evaluate the eggs (warm, hardboiled) produced by the experimental hens after peeling them and cutting them in half. Eggs from the control and treatment hens were randomly placed in covered plastic dishes and labelled using random numbers. The questionnaires given the panellists were constructed following the instructions proposed by Gonzalez-Esquerra and Leeson (2000), and used the same attributes and definitions: aroma, taste, aftertaste, flavour, presence of off-flavours and overall-acceptability. The panellists were asked to score their dislikes or likes (0 to 10 on a 10 point scale) for aroma, taste, aftertaste, flavour and acceptability, as well as the intensity of off-flavours (absent = 0, very strong = 10). When this scale is used in the evaluation of n-3 enriched commercial eggs, scores below 4 are deemed as «not acceptable», 5 to 7 is «acceptable» and above 8 is «normal». The eggs were presented to the panellists under red lighting to avoid any bias associated with yolk colour. Unsalted crackers and water were offered to cleanse the palate between samples.

The firmness of the hardboiled yolks of six eggs from each treatment was evaluated using a Texture Analyser TA-XT2 and Expert software (v. 2.61a) (Texture Technologies Corp., Scarsdale, NY). The tested eggs were stored for 21 days at 5ºC. They were then allowed to attain room temperature and were cooked in boiling water for 15 min. The cooked eggs were cooled to room temperature and the yolks separated from the shell and albumen. To determine the hardness of the yolks, the Texture Analyser was equipped with a TA-18 mm compression plate, and the resistance measured as the force (N) needed to compress the yolks to a distance of 6 mm between parallel planes. This distance was selected since it represented 20% of the original yolk height (Ahn *et al*., 1999).

Data were analysed as a completely randomised experimental design using the SAS software GLM procedure (SAS Institute Inc., 1990). The effects of the dietary supplementation level of FO and HOSO and their interaction were the main factors studied. Non-orthogonal contrasts were made to test the effect of incorporating 3 g CLA kg^{-1} to the control diet without added FO. Total dietary fat supplementation was included in the model as a covariate for yolk CLA concentrations. Panellist scores were included as a block for sensorial evaluation traits. Regression procedures (SAS Institute Inc., 1990) were used to predict the retention of CLA isomers in yolk fat as well as the overall acceptability of the eggs.

Results

The treatments did not affect either feed intake, laying rate, egg weight, shell thickness, albumen height or yolk colour; the mean values for these variables were 113 g (±3.11, SE), 89.8% (±5.50), 65.5 g (±2.91), 353 µm (± 11.5), 7.43 mm (± 0.59) and 12.5 (± 0.17), respectively. Supplementation with FO or HOSO affected neither the proportion of yolk nor albumen with respect to total egg weight, yolk or albumen pH, nor yolk or albumen moisture; the mean values obtained were 267 g kg^{-1} (±8.23), 640 g kg^{-1} (±9.84), 6.02 (±0.049), 9.04 (±0.078), 495 g kg^{-1} (±3.88) and 888 g kg^{-1} (± 3.04) respectively [not significantly different ($P > 0.15$) compared to values obtained with the control diet].

The type of diet did not affect the total yolk fat content, which averaged 329 g kg^{-1}, but it greatly influenced the egg yolk FA profile (Table 3). The addition of 3 g CLA kg^{-1} to the control diet with no added FO but with 30 g HOSO kg^{-1} increased ($P < 0.001$) the yolk fat concentrations of c_9,t_{11}, t_{10},c_{12}, $t_{11},t_{13} + t_{10},t_{12} + t_9,t_{11} + t_8,t_{10}$ CLA (tt CLA) by up to 6.32, 1.56, 1.14 and 9.02 g kg^{-1} respectively. The inclusion of CLA also increased ($P < 0.001$) the yolk fat content of $C_{16:0}$, $C_{18:0}$ and total saturated FA (SFA) by 21, 53 and 30% respectively, but reduced those of $C_{16:1}$, $C_{18:1}$ and total monounsaturated FA (MUFA) by 41, 19 and 21%. Dietary CLA supplementation also tended to increase the yolk concentration of $C_{18:2\ n\text{-}6}$ (by 16%, $P = 0.06$), $C_{18:3\ n\text{-}3}$ (by 26%, $P = 0.02$) and $C_{22:5\ n\text{-}3}$ (by 236%, $P = 0.06$), but to reduce that of $C_{20:4\ n\text{-}6}$ (by 23%, $P < 0.001$) and $C_{22:6\ n\text{-}3}$ (by 30%, $P = 0.10$). Yolk fat contents of $C_{20:5\ n\text{-}3}$, $C_{22:4\ n\text{-}6}$, total n-3 and total n-6 FAs were not affected.

Supplementation with 17 g FO kg^{-1} of diets containing 3 g CLA kg^{-1} decreased ($P < 0.05$) the yolk content of t_{10},c_{12} and tt CLA by 0.15 and 0.2 g kg^{-1} respectively, but not the c_9,t_{11} or total CLA contents. Fish oil supplementation had little influence on SFA and MUFA concentrations, although it slightly reduced $C_{18:0}$ and total saturated FAs by 7% and 5% respectively. The inclusion of FO in the diet did not affect $C_{18:2\ n\text{-}6}$ yolk fat concentration, greatly reduced

Table 3. Effect of the different diets on the egg yolk fatty acid profile (g kg^{-1} of total fatty acids)

Treatments	T1	T2	T3	T4	T5	Contrasts[1]				
						SEM	1	2	3	4
CLA supplement, g kg^{-1}	0	3	3	3	3					
HOSO supplement, g kg^{-1}	30	30	30	35	35					
FO supplement, g kg^{-1}	0	0	17	0	17					
Total fat content, g kg^{-1}	334	332	324	324	325	3.52	NS[3]	NS	NS	NS
$C_{16:0}$	233	281	269	271	270	4.64	0.001	NS	NS	NS
$C_{16:1\ n-7}$	27.2	16.0	17.1	14.8	16.0	0.80	0.001	NS	NS	NS
$C_{18:0}$	86.2	132	124	143	122	4.07	0.001	NS	0.005	NS
$C_{18:1\ n-9}$	503	403	397	405	404	4.96	0.001	NS	NS	NS
$C_{18:2\ n-6}$	102	118	111	112	108	4.89	0.06	NS	NS	NS
c_9,t_{11} CLA	0	6.32	6.48	6.18	6.16	0.18	0.001	NS	NS	NS
t_{10},c_{12} CLA	0	1.56	1.34	1.52	1.42	0.063	0.001	NS	0.04	NS
tt CLA[2]	0	1.14	0.84	1.14	0.84	0.071	0.001	NS	0.02	NS
$C_{18:3\ n-3}$	1.86	2.34	2.60	2.22	2.48	0.12	0.02	NS	0.06	NS
$C_{20:4\ n-6}$	18.0	13.8	8.68	15.2	7.96	0.58	0.001	NS	0.001	0.07
$C_{20:5\ n-3}$	0.10	0.16	1.22	0.20	1.00	0.052	NS	NS	0.001	0.07
$C_{22:4\ n-6}$	1.92	1.82	1.04	1.60	0.96	0.085	NS	0.06	0.001	NS
$C_{22:5\ n-3}$	0.28	0.94	3.72	0.82	3.20	0.19	0.06	NS	0.001	NS
$C_{22:6\ n-3}$	5.34	3.72	39.0	3.74	35.0	0.76	0.10	0.04	0.001	0.04
Total CLA	0	9.02	8.66	8.84	8.42	0.28	0.001	NS	NS	NS
Total SFA	323	419	401	420	400	4.73	0.001	NS	0.03	NS
Total MUFA	535	423	420	424	426	5.03	0.001	NS	NS	NS
Total n-3 FA	7.58	7.16	46.5	6.98	41.7	0.94	NS	0.04	0.001	0.05
Total non-CLA n-6 FA	122	133	121	129	117	4.69	NS	NS	0.04	NS

SEM: standard error of means (n = 5). SFA: total saturated fatty acids. MUFA: total monounsaturated fatty acid. NS: not significant (P > 0.10). [1] Contrasts: 1: effect of inclusion of CLA (T1 *vs* T2); 2: effect of inclusion of HOSO [(T2 + T3) *vs* (T4 +T5)]; 3: effect of inclusion of FO [(T2 + T4) *vs* (T3 + T5)]; 4: effect of the interaction HOSO * FO [(T3-T2) *vs* (T5-T4)]. [2] $t_{11},t_{13} + t_{10},t_{12} + t_9,t_{11} + t_8,t_{10}$ CLA. [3] NS: not significant (P > 0.10).

(P < 0.001) those of $C_{20:4\ n-6}$ and $C_{22:4\ n-6}$, and increased (P < 0.001) those of $C_{20:5\ n-3}$, $C_{22:5\ n-3}$, $C_{22:6\ n-3}$ and total n-3 FA from 0.18, 0.88, 3.73 and 7.07 to 1.11, 3.46, 37.0 and 44.1 g kg^{-1} respectively.

Increasing the HOSO supplement from 30 to 35 g kg^{-1} in diets containing 3 g CLA kg^{-1} did not significantly affect yolk CLA, SFA or MUFA concentrations (including $C_{18:1}$), and had little effect on the n-6 and n-3 FA concentrations. Yolk contents of long chain polyunsaturated FA (PUFA) $C_{22:4\ n-6}$ and $C_{22:6\ n-3}$ tended to decrease by about 10% with increasing HOSO supplementation.

The interaction between FO and HOSO had a significant (P < 0.07) effect on the $C_{20:4\ n-6}$, $C_{20.5\ n-3}$, $C_{22:6\ n-3}$ and total n-3 FA yolk contents; the effect of including 17 g kg^{-1} of FO on these traits was smaller when the amount of HOSO in the diet was increased from 30 to 35 g kg^{-1}.

Table 4 shows the effects of the different treatments on egg sensorial quality. The addition of 3 g CLA kg^{-1} to diets containing 30 g HOSO kg^{-1} but with no added FO, impaired (P < 0.05) the aroma, taste, aftertaste, flavour and acceptability of hardboiled eggs. Neither the presence of off-flavours nor yolk firmness were modified by the addition of CLA.

Supplementation with 17 g kg^{-1} of FO of diets containing 3g CLA kg^{-1} plus 30-35 g HOSO kg^{-1} did not influence yolk firmness but had a negative effect (P < 0.001) on all the egg sensorial traits evaluated. The eggs produced were considered unacceptable for human consumption by the evaluation panel.

Neither increasing HOSO from 30 to 35 g kg^{-1} nor the interaction between FO and HOSO significantly affected any of the sensorial traits studied.

Discussion

The type of diet had little effect on hen performance. However, the length of experimental period was short

Table 4. Effect of the different diets on the sensorial and rheological properties (yolk firmness) of hardboiled eggs[1]

Treatments	T1	T2	T3	T4	T5	Contrasts[2]				
						SEM	1	2	3	4
CLA supplement, g kg^{-1}	0	3	3	3	3					
HOSO supplement, g kg^{-1}	30	30	30	35	35					
FO supplement, g kg^{-1}	0	0	17	0	17					
Aroma	7.67	6.25	4.67	6.00	4.50	0.42	0.05	NS[3]	0.005	NS
Taste	8.25	6.42	2.25	6.50	2.25	0.49	0.008	NS	0.001	NS
Aftertaste	8.08	6.17	2.42	6.42	2.33	0.46	0.006	NS	0.001	NS
Flavour	8.00	6.17	2.50	6.50	2.50	0.47	0.006	NS	0.001	NS
Off-flavours	1.00	2.83	6.33	2.25	5.92	0.66	NS	NS	0.001	NS
Acceptability	7.75	5.75	2.50	6.33	2.83	0.52	0.01	NS	0.001	NS
Yolk firmness, N	5.17	5.33	4.67	4.67	4.17	0.48	NS	NS	NS	NS

SEM: standard error of means (n = 12, except for yolk firmness, where n = 6). [1] Range: disliked = 0, liked = 10, for aroma, taste, aftertaste, flavour and acceptability, and from absent = 0 to very strong = 10 for off-flavours. [2] Contrasts: 1: effect of inclusion of CLA (T1 *vs* T2); 2: effect of inclusion of HOSO [(T2 + T3) *vs* (T4 +T5)]; 3: effect of inclusion of FO [(T2 + T4) *vs* (T3 + T5)]; 4: effect of the interaction HOSO * FO [(T3-T2) *vs* (T5-T4)]. [3] NS: non significant (P > 0.10)

(seven weeks) and the number of replicates small (five per treatment); it may therefore be premature to conclude the lack of any effect of these treatments on production traits. Previous work has shown that CLA supplementation at higher levels for longer periods leads to a reduction in the weight gain of hens (Jones *et al.*, 2000; Álvarez *et al.*, 2004a).

The present results show that combined supplementation with 3 g kg^{-1} CLA and 30 g kg^{-1} HOSO leads to the production of eggs containing 9 g CLA per kg of yolk fat. This impaired several sensorial quality traits compared to eggs produced by hens receiving the control diet with no added CLA, although they were classed as acceptable by the panellists (above 5.75 on a scale from 0 to 10). The inclusion of 3 g CLA kg^{-1} increased the degree of saturation of the FAs in the yolk fat, but the simultaneous addition of 30 g kg^{-1} HOSO allowed a high yolk $C_{18:1}$ content (403 g kg^{-1}) to be maintained and the firmness of the yolks was similar to that of control eggs. Other characteristics related to low consumer acceptance, such as changes in yolk and albumen pH or yolk moisture (Ahn *et al.*, 1999; Du *et al.*, 1999; 2000; Álvarez *et al.*, 2004b, 2005), were not affected by the addition of CLA to the control diet.

In the present study, the average yolk CLA concentration obtained in diets supplemented with 3 g CLA kg^{-1} (8.74 g kg^{-1}) was within the 3 to 22 g kg^{-1} range found in ruminant products (meat/milk etc.) (Chin *et al.*, 1992; Dhiman *et al.*, 1999). It was also higher than that previously obtained (6.82 g kg^{-1}) in eggs from hens fed diets containing 2 g kg^{-1} of CLA and 30 g kg^{-1} of HOSO (Álvarez *et al.*, 2005), although the sensorial evaluation was slightly worse.

Linoleic conjugated acid was retained in the yolk fat with an average efficiency of 13%. This tended to decrease (P = 0.10) with the level of fat supplementation of the basal diet. This agrees with previous observations (Raes *et al.*, 2002; Álvarez *et al.*, 2004 b, 2005), in which lower levels of total fat supplementation of the basal diet were provided (Álvarez *et al.*, 2004 a,b, 2005).

The retention of isomer c_9,t_{11} was greater than that of t_{10},c_{12}, as recorded in previous studies (Du *et al.*, 1999; Jones *et al.*, 2000; Aydin *et al.*, 2001; Raes *et al.*, 2002; Álvarez *et al.*, 2004 a,b, 2005), in which this was related to differences in the rate of its catabolism. Using the data of the present work plus those published by Álvarez *et al.* (2004a,b, 2005) [which were recorded using a similar methodology and under similar experimental conditions (n = 26)], a regression analysis was performed in order to predict the concentration of both isomers in the yolk fat of eggs from CLA-supplemented hens. Figure 1 shows the equations obtained. These indicate a linear and quadratic effect (P < 0.001) of dietary CLA content on the retention of both isomers, and a higher rate of deposition (around double) for c_9,t_{11} than for t_{10},c_{12} CLA. The results of the present study also suggest that some tt- CLA isomers were formed in the hens from c9,t11 or t10,c12 dietary CLA, since tt isomers were not detected in the CLA source.

Dietary supplementation with CLA did not interfere with the synthesis of total long-chain n-3 FA since the

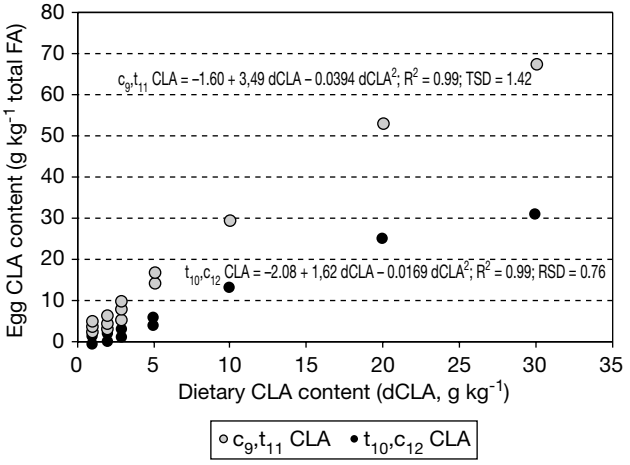

Figure 1. Effect of CLA supplementation (dCLA, g kg^{-1}) on egg c_9,t_{11} and t_{10},c_{12}-CLA concentrations (g kg^{-1} of total fatty acids) according to different studies.

increasing retention of $C_{22:5\ n-3}$ was compensated for by a decreasing trend for $C_{22:6\ n-3}$. A positive relationship between CLA addition and $C_{22.5\ n-3}$ yolk retention is also reported by Raes *et al*. (2002) and Álvarez *et al*. (2004b). Dietary supplementation with CLA reduced yolk retention of $C_{20:4\ n-6}$, which suggests that CLA favours the use of elongases and desaturases for n-3 rather than for n-6 long-chain FA metabolism, as reported by Álvarez *et al*. (2004b).

Increasing the supplement of HOSO from 30 to 35 g kg^{-1} had little effect on yolk fat concentrations of CLA or other FAs, including $C_{18:1}$. Nor did it significantly affect the sensorial quality of the eggs laid. Álvarez *et al.* (2005) reported a reduction in yolk CLA concentration, an increase in yolk $C_{18:1}$ content, and improved sensorial quality following an increase in HOSO supplementation from 10 to 30 g kg^{-1}. Similar trends were observed in this work, although the effects did not reach statistical significance due to the smaller variation in dietary $C_{18:1}$ content among treatments. According to the present results, high levels of dietary $C_{18:1}$ supplementation might lead to a reduction in the efficiency of retention of $C_{18:1}$ in yolk fat, and to reduced elongase and Δ4 desaturase activity [enzymes required for the synthesis of long-chain n-6 and n-3 FA ($C_{22:4\ n-6}$ and $C_{22:6\ n-3}$)].

Dietary supplementation with 17 g FO kg^{-1} led to an increase in the yolk fat concentration of long-chain n-3 FAs, especially $C_{22:6\ n-3}$. The efficiency of retention for total n-3 FAs was close to 50%, decreasing at the higher HOSO supplementation level. Supplementation with FO reduced the yolk retention of long-chain n-6 FA, reflecting a reduced availability of the enzymes required for its synthesis (these are shared with the n-3 FA series). The addition of FO had an overall positive effect on yolk PUFA content (by 18%, $P < 0.05$) and tended to reduce the firmness of hardboiled eggs, although the differences did not reach statistical significance.

The inclusion of FO in diets with CLA and HOSO supplements greatly impaired the sensorial quality of the eggs produced; these were deemed unacceptable for human consumption by the panellists (values < 5), although the level of FO supplementation was close to that used in the commercial production of n-3 enriched eggs.

An excess of dietary FO or other sources of n-3 FA, such as flaxseed, has long been recognized to reduce egg sensorial quality (Van Elswyk, 1997; Noble, 1998; González-Esquerra and Leeson, 2001; Surai and Sparks, 2001). It has been suggested that adverse flavours are promoted by oxidative deterioration of the egg lipids. Long-chain n-3 FAs are highly susceptible to peroxidation because of their long length and many double bonds. The inclusion of marine algal oil in the diet seems to have less of a negative effect on sensorial scores despite its high $C_{22:6\ n-3}$ concentration. This has been related to its high natural carotenoid content which might help to stabilize yolk lipids (Herber-McNeill and Van Elswyk, 1996, 1998). In the present study, the antioxidant properties of CLA might have helped to reduce the negative effect of FO addition, but failed to produce eggs double-enriched in CLA and n-3 FA that were of acceptable sensorial quality. Similarly, the supplementation of diets with high levels of vitamin E (100 IU kg^{-1}) and a high flaxseed content (200 g kg^{-1}) results in a further reduction of overall egg acceptability (Leeson *et al.,* 1998). Deodorization of FO to reduce its content of volatile compounds produced via lipid oxidation neither appear to improve it (González-Esquerra and Leeson, 2000). The reasons for the poorer acceptability of eggs enriched in long-chain n-3 FA and/or CLA therefore remain unclear.

Stepwise regression analysis was performed in order to relate overall egg acceptability (A, in a scale from 0 to 10) to yolk FA composition (g kg^{-1}), using data from this experiment and previous studies performed using the same methodology (Álvarez *et al.,* 2005 and unpublished data; n = 22). The method selected $C_{22:5\ n-3}$ yolk content as the first independent variable, probably because of its high correlation with the other n-3 FA ($r > 0.95$) and yolk CLA concentrations ($r = 0.647$):

$$A = 7.05 - 1.19\ C_{22:5\ n-3};\ R^2 = 0.891;\ RSD = 0.774;\ P < 0.001$$

In a second step, yolk CLA content was also included in the model:

$$A = 7.49 - 0.127\ CLA - 0.993\ C_{22:5\ n-3};\ R^2 = 0.926;\ RSD = 0.654;\ (P < 0.001)$$

Panellist score was also negatively ($P < 0.001$) correlated with yolk fat $C_{20:5\ n-3}$ content ($r = -0.86$); $C_{22:6\ n-3}$ ($r = -0.84$) and total n-3 FA ($r = -0.86$).

The greater influence of $C_{22:5\ n-3}$ than other n-3 FAs on the presence of off-flavours would also explain the lower impact on egg sensory quality of marine algae compared to FO, as the inclusion of marine algae in the diet leads to an increase in $C_{22:6\ n-3}$ retention in yolk content, whereas the concentration of $C_{22:5\ n-3}$ remains almost unchanged (Herber-McNeill and Van Elswyk, 1996; Cachaldora *et al*., unpublished).

The results of the present work indicate that the combined addition of appropriate levels of CLA and HOSO to the diet of laying hens allows the production of eggs with a moderate CLA content (9 g kg^{-1} yolk fat) and an acceptable sensorial quality. However, the study failed to obtain commercially viable eggs double-enriched in CLA and long-chain n-3 FA, since the negative effects of FO on sensorial quality were additive with those of CLA. Further research is needed to attain this objective by modifying the supplementation-induced levels of and/or source of n-3 FA.

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