Spanish Journal of Agricultural Research (2004) 2 (2), 203-209

Effects of conjugated linoleic acid addition on its deposition in eggs of laying hens, fed with no other fat source

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Abstract

An experiment was conducted to investigate the retention of conjugated linoleic acid (CLA) in eggs of laying hens by replacing 1, 2 or 3% of a control diet with no fat added with a commercial source of CLA. Sixty four 40-weeks-old Warren laying hens were used to determine the effect of treatments on productive traits, yolk fatty acids composition and egg quality. The data were collected over a 56-days experimental period, following a 21-days adaptation period. Type of diet did not affect feed intake, laying rate, egg or yolk weight, although weight gain of hens decreased linearly ($P < 0.001$) with level of CLA inclusion. Concentration of CLA in yolk fat increased with dietary CLA addition, reaching a value of 10.5% for the 3%-CLA diet. Efficiency of retention of CLA decreased linearly ($P < 0.001$) with level of CLA inclusion, from 24.1 to 18.6% between the extreme diets. An increase of dietary CLA led to an increase of saturated and a decrease of monounsaturated and n-3 polyunsaturated fatty acids ($P < 0.001$). These changes were greater at lower levels of CLA inclusion. Sensory evaluation showed that texture of eggs produced at any CLA level was not acceptable for consumption.

Key words: CLA, layers, performance, efficiency, fatty acids.

Resumen

Retención de ácido linoleico conjugado en huevos de gallinas ponedoras alimentadas con dietas sin otra fuente de grasa añadida

Se realizó un experimento para investigar la retención de ácido linoleico conjugado (ALC) en huevos de gallinas, reemplazando un 1, 2 ó 3% de una dieta control, sin grasa añadida, con una fuente comercial de ALC. Se utilizaron 64 gallinas Warren de 40 semanas de edad para determinar el efecto de los tratamientos sobre parámetros productivos, composición en ácidos grasos de la yema y calidad del huevo. Los datos se recogieron a lo largo de un período experimental de 56 días, después de un período de adaptación de 21 días. El tipo de dieta no afectó al consumo, al nivel de puesta, ni al peso del huevo o de la yema, pero la ganancia de peso de las gallinas se redujo linealmente ($P < 0,001$) con el nivel de inclusión de ALC. La concentración de ALC en la grasa de la yema aumentó con la adición de ALC a la dieta, alcanzando un valor de 10,5% en la dieta con un 3% de ALC. La eficacia de retención de ALC disminuyó linealmente ($P < 0,001$) con el nivel de inclusión de ALC, desde un 24,1 hasta un 18,6% en las dietas extremas. Un aumento del contenido de ALC en la dieta se tradujo en un incremento en la proporción de grasa saturada y en una disminución de la de ácidos grasos monoinsaturados y poliinsaturados n-3 ($P < 0,001$). Estos cambios fueron mayores en los niveles más bajos de suplementación con ALC. La valoración sensorial mostró que la textura de los huevos producidos a cualquier dosis de ALC no era aceptable para su consumo.

Palabras clave: ALC, rendimientos, eficacia, ácidos grasos.

Introduction

Linoleic conjugated acid (CLA) refers to a mixture of isomers of linoleic acid, that is produced in biohydrogenation processes, as those occurring in the rumen. Several studies (Ha *et al.*, 1990; Shultz *et al.*, 1992a,b; Lee *et al.*, 1994; Ip *et al.*, 1994; Pariza, 1997; Parodi, 1999) have demonstrated that relatively low dietary levels of CLA have health benefits (anticarcinogenic, antiatherogenic, antioxidant and antidiabetic) on laboratory animals. As a result, there has been

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Received: 26-11-03; Accepted: 11-02-04.

considerable interest in increasing CLA concentrations in animal feeds to provide healthful products for human consumption.

Previous work (Chamruspollert and Sell, 1999; Du *et al.*, 1999, 2000; Jones *et al.*, 2000; Aydin *et al.*, 2001; Schaffer *et al.*, 2001; Cherian *et al.*, 2002; Raes *et al.*, 2002; Szymczyck and Pisulewski, 2003) has shown that it is possible to produce CLA-enriched eggs through CLA supplementation of layer diets. The relationship between CLA concentrations in egg yolk and feed was almost linear up to levels of inclusion of CLA in the diet of 5%. Efficiency of retention of CLA was around 20%, but varied with type of CLA-isomer used (Du *et al.*, 1999; Jones *et al.*, 2000; Szymczyk and Pisulewski, 2003). Furthermore, in most of these studies CLA replaced different types of fat in the diet, so that the effects of inclusion of CLA were confounded and varied with the type and level of fat in the control diet. Consequently, accumulation of CLA in eggs might differ if CLA is included in the feed replacing a whole basal diet with no fat added.

Some of these studies also indicate that feeding CLA to hens led to undesirable changes of egg fat composition. The concentrations of monounsaturated (MUFA) and non-CLA polyunsaturated (PUFA) fatty acids were reduced, whereas that of saturated fatty acids (SFA) increased greatly. These effects have been attributed to a decreased activity of Δ9 desaturase and concurrent changes in dietary concentrations of fatty acids with CLA-supplementation (Chamruspollert and Sell, 1999), and were evident at dietary CLA levels as low as 0.5%. Variations in yolk fatty acid content might then differ if no fat from the control diet is replaced with CLA.

The objectives of this study were to determine the effects of supplementation with different levels of CLA (1, 2 or 3%) of a commercial diet with no fat added on the zootechnical performance of laying hens as well as on CLA and other fatty acid concentration and sensory properties of eggs.

Material and Methods

Sixty four 40-week-old Warren laying hens were individually housed in cages (33 × 41 cm) and served as the experimental unit. The trial lasted for 56 days and started after a pre-experimental period of 21 days. Feed was restricted at 115 g d^{-1} and water was supplied ad libitum. The hens received 15 h light d^{-1} throughout the experiment. Room temperature was also controlled at about 21ºC.

Hens were assigned at random to each of four dietary treatments, formulated to include 1, 2 or 3% of CLA into a basal diet with no fat added. The CLA source used in this study was obtained from BASF Española S.A. and contained 56% CLA. The actual amounts of CLA source added to the diets were therefore 1.8, 3.6 and 5.4%.

The ingredients and chemical composition of the basal diet are shown in Table 1. The fatty acids (FA) profiles of the experimental diets are shown in Table 2. The diets were also analysed (AOAC, 1990) to determine moisture by the oven-drying method (930.15), protein by the Kjeldahl method (984.13), and ether extract by Soxhlet fat analysis (920.39). All samples were analysed in duplicate. Yolk lipids were extracted following the method of Folch *et al.* (1957). Fatty acid profiles of experimental fats, diets, and egg yolks were determined according to the methods of Cherian and Sim (1992). The fat extracted from each sample was methylated (Metcalfe *et al.*, 1961), and the FA were separated and identified using a Hewlett-Packard 5890 gas chromatograph (Varian; Walnut Creek, California,

Table 1. Ingredient and chemical composition of the control diet (% as fed basis)

Ingredients
— Corn grain: 30.8
— Wheat grain: 32.0
— Soybean meal 44%: 17.5
— Soybean meal 47%: 8.12
— Calcium carbonate: 9.75
— Dicalcium phosphate: 1.04
— Sodium chloride: 0.29
— Formic acid: 0.25
— Microingredients[1]: 0.25
— Calculated analysis[2], %
— AMEn, kcal kg^{-1}: 2583
— Crude protein: 17.5
— Lysine: 0.88
— Methionine: 0.28
— Methionine + Cystine: 0.60
— Ether extract: 2.11
— Calcium: 4.08

[1] Provided the following per kilogram of diet: vitamin A, 10000 IU; cholecalciferol, 2200 IU; vitamin E, 13 IU; menadione sodium bisulfite, 2.0 mg; riboflavin, 4 mg; D-calcium pantothenate, 8 mg; nicotinic acid, 28 mg; pyridoxine hydrochloride, 0.8 mg; folic acid, 0.25 mg; d-biotin, 0.05 mg; thiamine hydrochloride, 1 mg; vitamin B12, 10 µg; choline chloride, 250 mg; Mn, 80 mg; Zn, 65 mg; Fe, 40 mg; Cu, 8 mg; I, 1.9 mg; Se, 0.25 mg; lutein, 6 mg; canthaxantin, 3 mg; ethoxiquin, 125 mg.
[2] According to FEDNA (2003).

Table 2. Analysed fatty acid profile of the ether extract of the experimental diets (% of total fatty acids)

Fatty acid	Supplemental CLA (%)			
	0	1	2	3
$C_{16:0}$	14.44	10.94	9.90	8.56
$C_{16:1\ n-7}$	0.26	0.21	0.23	0.16
$C_{18:0}$	3.21	3.83	4.28	4.12
$C_{18:1\ n-9}$	23.66	23.16	23.80	23.12
$C_{18:2\ n-6}$	53.42	31.48	23.31	19.07
$C_{18:3\ n-3}$	3.27	1.95	1.39	1.12
$C_{20:5\ n-3}$	0	0.06	0.09	0.10
$C_{22:6\ n-3}$	0	0	0.01	0.01
c9,t11 CLA	0	13.24	17.31	20.70
t10,c12 CLA	0	12.71	16.79	20.12
ccCLA[1]	0	0.49	0.74	0.82
ttCLA[2]	0	0.86	1.24	1.15
Total CLA	0	27.30	36.08	42.79
Total SFA[3]	17.65	14.82	14.25	12.76
Total MUFA[4]	23.92	23.37	24.03	23.28
Non CLA-PUFA[5]	56.69	33.43	24.70	20.19

[1] c8,c10 + c9,c11 + c10,c12 + c11,c13 CLA. [2] t11,t13 + t10,t12 + t9,t11 + t8,t10 CLA. [3] Total saturated fatty acids. [4] Total monounsaturated fatty acids. [5] Total non-CLA polyunsaturated fatty acids.

USA) equipped with a Supelco SP-2330 (30 m × 0.25 mm inside diameter) capillary column of silica. 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. Calibration and identification of the peak for the different FA were obtained by comparing the retention time with that of a standard (Qualimix Fich S. Ref: 89-5550 Larodan; Malmoe, Sweden) whose composition was previously known.

Hen-day egg production and feed consumption were measured daily throughout the trial. Body weights of the hens were measured at the beginning and at the end of the experimental period. The last three eggs produced by each hen at the end of experimental period were kept to analyze yolk FA composition, and the average values were used for statistical analysis. Additionally, six extra eggs per replicate were collected during the last period. Three of them were used to determine commercial value on the basis of Haugh units, shell thickness, and yolk color as measured by the Roche yolk color fan (Vuilleumier, 1969). The other three eggs were used to determine the weights of yolk, albumen, and shell plus membranes, according to the procedure described by Hussein *et al.* (1992).

Sensory evaluation was conducted with hardboiled eggs kept at 5ºC for 14 days to facilitate peeling. Eggs were evaluated by twelve trained panellists to test them for aroma and taste and to detect off flavours using a nine point score scale.

The effects of inclusion of CLA in the basal diet was analysed by ANOVA with a completely randomised design by using the SAS software (SAS Institute Inc., 1990). Treatment sums of squares were partitioned into linear and quadratic effects by using orthogonal contrasts. Mean comparisons were made by using a t-test.

Results

The effect of treatments on productive traits and egg quality is shown in Table 3. No feed refusals were

Table 3. Effect of dietary CLA inclusion on production performance of laying hens and commercial quality of eggs

Production trait	Supplemental CLA (%)				SEM[1]	P
	0	1	2	3		
Hen-day production (%)	89.8	90.2	85.3	91.7	2.7	0.19
Egg weight (g)	62.5	63.5	62.0	62.6	1.0	0.58
Weight gain (g)[2,3]	16.7^{a}	$-45.8^{a,b}$	-70.8^{b}	-100^{b}	27.1	0.01
Yolk weight (g)	15.7	16.0	15.4	15.2	0.30	0.23
Albumen height (mm)[4]	$6.34^{a,b}$	$6.80^{a,b}$	7.17^{a}	5.98^{b}	0.39	0.05
Haugh units[5]	$75.1^{b,c}$	$80.5^{a,b}$	82.0^{a}	73.1^{c}	2.8	0.02
Yolk colour[6,7]	12.1^{a}	12.7^{b}	$12.4^{a,b}$	12.7^{b}	0.20	0.01
Shell thickness (mm)	348	353	342	358	7.4	0.28

[a,b,c] Means with different superscripts within the same row differ ($P < 0.05$). [1] Standard error of means (n = 16). [2] Over the whole experimental period (56 days). [3] Linear effect of CLA level of inclusion ($P = 0.001$). [4] Quadratic effect of CLA level of inclusion ($P = 0.01$). [5] Quadratic effect of CLA level of inclusion ($P = 0.002$). [6] Linear effect of CLA level of inclusion ($P = 0.02$). [7] Roche colour fan (Vuilleumier, 1969).

observed, thus the type of diet did not affect either feed intake, hen-day production or egg and yolk weight. Dietary CLA level affected quadratically ($P < 0.01$) both albumen height and Haugh units, which showed maximal values for the diet containing 2% of CLA. Yolk colour increased ($P < 0.05$) in hens fed diets supplemented with CLA with respect to hens fed the control diet. An increase of dietary level of inclusion of CLA led to a linear ($P < 0.001$) decrease of weight gain, so that hens fed on the control diet were on positive weight balance (16.7 g), whereas those fed the 3%-CLA diet lost 100 g (a 5% of its average body weight) over the whole experimental period (56 days).

Treatments did not affect total ether extract concentration but greatly influenced fatty acid profile of egg yolk as shown in Table 4. An increase of dietary CLA supplementation increased ($P < 0.001$) all the CLA isomers contents. Total egg CLA concentration increased up to 10.5% in the 3%-CLA diet. Concurrent changes in the profile of other fatty acids were also observed. Palmitic, estearic, and total saturated fatty acid contents also increased ($P < 0.001$) with CLA inclusion by 20, 112 and 45%, respectively, between extreme diets. Instead, yolk fat concentrations of palmitoleic, oleic, erucic and total monounsaturated fatty acids decreased, respectively by 87, 54, 50 and 56%. However, only slight changes in polyunsaturated fatty acid concentrations were observed. Most of the effects were both linear and quadratic, indicating that the magnitude of the differences for each 1% of dietary CLA increment decreased with CLA level of inclusion.

Efficiency of CLA retention in egg yolk was determined as the proportion of total g CLA in eggs/total g CLA ingested daily. Results showed a linear decrease ($P < 0.001$) of this efficiency with CLA level of inclusion, i.e. 0.241, 0.211 and 0.186 (SEM = 0.011) for 1, 2 and 3% CLA-diets, respectively.

Sensory evaluation tests showed that eggs produced by hens fed CLA supplemented diets were not acceptable for consumption. The textures of boiled CLA-eggs were rubbery and elastic. Furthermore, firmness of the yolk increased with level of inclusion of CLA in the diet.

Table 4. Effect of dietary CLA inclusion on fatty acid profile of egg yolk (% of total fatty acids)

Fatty acid	Supplemental CLA (%)				SEM[1]	P
	0	1	2	3		
Total fat (%)	32.7	32.3	32.2	32.0	0.28	0.43
$C_{16:0}$[2]	28.1[c]	35.9[a]	34.5[b]	33.7[b]	0.35	0.001
$C_{16:1\ n-7}$[2]	3.04[a]	1.00[b]	0.539[c]	0.409[c]	0.11	0.001
$C_{18:0}$[2]	10.3[c]	20.9[b]	22.3[a]	21.8[a]	0.32	0.001
$C_{18:1\ n-9}$[2]	41.3[a]	21.6[b]	18.9[c]	18.9[c]	0.35	0.001
$C_{18:2\ n-6}$[3]	11.3[a]	11.2[a,b]	10.9[a,b]	10.6[b]	0.23	0.10
$C_{18:3\ n-3}$[4]	0.394[c]	0.459[a]	0.438[a,b]	0.411[b,c]	0.012	0.001
$C_{22:1\ n-9}$[2]	1.68[a]	1.11[b]	0.926[c]	0.849[c]	0.03	0.001
$C_{20:5\ n-3}$[3]	0.0089[b]	0.015[a,b]	0.021[a]	0.024[a]	0.0035	0.012
$C_{22:6\ n-3}$[2]	0.624[a]	0.302[b]	0.198[c]	0.164[c]	0.025	0.001
c9,t11 CLA[2]	-	2.96[c]	5.30[b]	6.73[a]	0.10	0.001
t10,c12 CLA[2]	-	1.31[c]	2.48[b]	3.08[a]	0.06	0.001
ccCLA[2,5]	-	0.061[c]	0.114[b]	0.169[a]	0.0075	0.001
ttCLA[2,6]	-	0.341[b]	0.490[a]	0.519[a]	0.013	0.001
Total CLA[2]	-	4.67[c]	8.39[b]	10.5[a]	0.16	0.001
Total SFA[2,7]	38.7[c]	57.5[a]	57.5[a]	56.1[b]	0.48	0.001
Total MUFA[2,8]	46.0[a]	23.7[b]	20.4[c]	20.1[c]	0.40	0.001
Non CLA-PUFA[3,9]	12.4[a]	12.0[a,b]	11.6[b,c]	11.2[c]	0.25	0.001

[a,b,c] Means with different superscripts within the same row differ ($P < 0.05$). [1] Standard error of means (n = 16). [2] Linear and quadratic effect of CLA level of inclusion ($P = 0.001$). [3] Linear effect of CLA level of inclusion ($P = 0.01$). [4] Quadratic effect of CLA level of inclusion ($P = 0.001$). [5] c8,c10 + c9,c11 + c10,c12 + c11,c13 CLA. [6] 6t11,t13 + t10,t12 + t9,t11 + t8,t10 CLA. [7] Total saturated fatty acids. [8] Total monounsaturated fatty acids. [9] Total non-CLA polyunsaturated fatty acids.

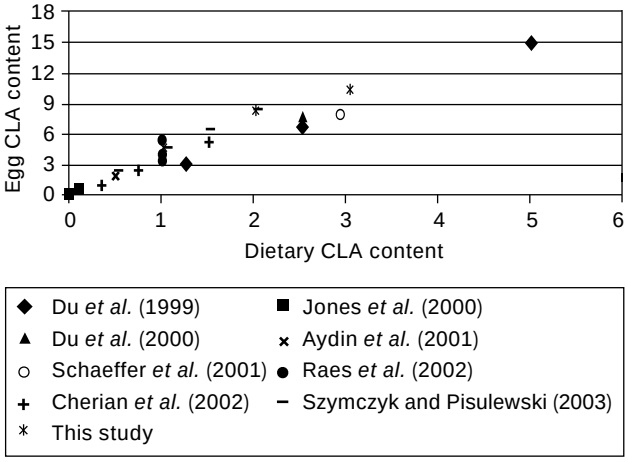

Figure 1. Effect of dietary CLA inclusión (% total ether extract) on egg-CLA concentration (% total ether extract) according to several studies.

Discussion

The results of this study confirms previous work that has established the possibility of producing CLA-enriched eggs through dietary supplementation. However, as shown in Fig. 1, egg CLA concentrations obtained were generally above those observed in other work at similar levels of CLA in the diet. This result might indicate a higher efficiency of CLA retention when no other fats are included in the feed. Likewise, Raes *et al.* (2002) found higher CLA concentrations in eggs produced by hens fed with low fat diets than those obtained from diets supplemented with soybean oil, animal fat or fish oil. Likewise, total CLA content was 4.67% of total FA in 1%-CLA diet, a higher value than that found in the main natural sources of CLA (milk fat and ruminant meat) i.e. from 0.3 to 2.2% (Chin *et al.*, 1992; Dhiman *et al.*,1999). Data in Table 4 also show a higher deposition in eggs of CLA isomer c9,t11 than that of t10,c12, although concentrations of both isomers in the experimental diets were similar (see Table 2), which agrees with previous studies (Du *et al.*, 1999; Jones *et al.*, 2000; Raes *et al.*, 2002). This difference might be explained by a higher efficiency of retention of c9,t11 CLA, as Park *et al.* (1999) have reported that t10,c12 CLA is catabolized more rapidly.

The increase in SFA (especially palmitic and estearic acids) and the decrease of MUFA (mainly palmitoleic and oleic acids) yolk concentrations with dietary CLA inclusion has also been described by other authors (Chamruspollert and Sell, 1999; Aydin *et al.*, 2001; Schaffer *et al.*, 2001; Cherian *et al.*, 2002; Raes *et al.*, 2002; Szymczyk and Pisulewski, 2003), although variations were quantitatively higher in the present study. These changes have been related to an inhibition of Δ9-desaturase with CLA supplementation, as this enzyme is responsible for the metabolic conversion of palmitic and estearic in palmitoleic and oleic acids, respectively. Inclusion of CLA in the diet also implied a decrease of docosahexanoic (C22:6 n-3, DHA) acid yolk concentration. This effect might be related to a parallel decrease in dietary linolenic acid content (C18:3 n-3, see Table 2), which is the main metabolic substrate for its synthesis. Therefore, all these changes in FA profiles, other than those affecting CLA, should be considered as inadequate for human nutrition, given the current dietary recommendations on FA consumption.

Treatments had little influence on zootechnical performance, egg or yolk weight, but dietary inclusion of CLA affected linear and negatively weight gain of hens. A similar but smaller effect was observed by Jones *et al.* (2000) when supplementing diets with lower CLA doses (up to 0.1%) than those used in our study. Body weight decreased significantly (by 5%) when comparing extreme diets throughout the whole experimental period (56 days), a result that might have practical importance if high CLA doses were used throughout long periods of time. Work made on growing pigs and rabbits (Corino *et al.*, 2001 and 2002; Swan *et al.*, 2001; Thiel-Cooper *et al.*, 2001; Wiegand *et al.*, 2001) has also shown a decrease of fat retention with CLA supplementation. Also, yolk colour increased when CLA was added to the diet, which might reflect a higher oxicarotenoids absorption in diets with higher ether extract content.

Inclusion of CLA impaired sensory quality of eggs in this study. Other work has also shown that dietary CLA supplementation (from 0.5% onwards) altered yolk texture (to rubbery and elastic) of hard-boiled eggs (Ahn *et al.*, 1999; Aydin *et al.*, 2001). These changes were parallel to an increase of yolk water content and yolk pH, a decrease of albumen pH, and movement of ions through yolk membrane during storage. These effects have been related to the incorporation of CLA into the phospholipids of the yolk membrane (Du *et al.*, 1999 and 2000), increasing its permeability.

These results suggest that efficiency of deposition of CLA in yolk fat can be increased by feeding diets with no other fat source. Future research should be focused on lower rates of CLA supplementation to reduce its negative effects on long-term variations of body reserves and egg quality.

Acknowledgments

This work was financed by INIA Project CAL-02-058.

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