



Effect of oleic and conjugated linoleic acid in the diet of broiler chickens on the live growth performances, carcass traits and meat fatty acid profile

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ABSTRACT

Olive oil and CLA enriched olive oil were compared with each other in a growth trial with broiler chickens, as energy supplements to the diet. A commercial CLA blend was used at the level of 1 kg per 100 kg mixed integrated feed.

Two hundred and forty commercial hybrid broilers (Ross 308) were randomly subdivided and allotted to 8 pens of 30 birds each. Four pens of birds were fed the olive oil diet and considered the control group; the other 4 pens were fed the olive oil supplemented with CLA and considered the treated group. The experiment lasted 47 days.

The live performance of the treated birds resulted different from the performance of the control ones: the final body weight was slightly lighter (2.544 kg vs 2.639 kg; $P \leq 0.05$) with a lower feed intake (4.886 kg feed vs 4.998 kg, $P \leq 0.05$) and, of course, an almost perfectly overlapping feed/gain ratio (1.90 vs 1.91).

The fatty acid composition of the breast fat of the CLA treated birds resulted enriched by the two major CLA isomers, trans 10 cis 12 and cis 9 trans 11, whereas oleic acid and the linoleic, linolenic and arachidonic polyunsaturated acids showed a decrease ($P \leq 0.05$).

CLA appears a recommendable ingredient in the diets of broilers as it improves the beneficial characteristics of poultry meat.

Key words: Broilers, CLA, Oleic acid, Meat quality.

RIASSUNTO

EFFETTI DEL CONTENUTO IN ACIDO OLEICO ED ACIDO LINOLEICO CONIUGATO NELLE DIETE PER BROILER SUL PROFILO IN ACIDI GRASSI DELLA CARNE

Alcuni studi hanno messo in evidenza che l'acido linoleico coniugato (CLA) presente nella dieta può influenzare la deposizione del grasso corporeo e il profilo in acidi grassi della carne. In particolare, un au-

mento del contenuto di CLA in diete destinate all'alimentazione del pollo sembra essere correlato ad una diminuzione di acido Oleico (OA) nella carne di questi animali. In letteratura sono ben note le proprietà nutraceutiche di entrambe queste molecole delle quali la prima è caratteristica del grasso degli alimenti provenienti dall'allevamento dei ruminanti, mentre la seconda dell'olio di oliva. L'obiettivo di questo lavoro è stato quello di verificare la possibilità di modificare il profilo in acidi grassi della carne di pollo, aumentandone contemporaneamente la percentuale di CLA ed OA, attraverso l'alimentazione. Per realizzare questo scopo, 240 broiler (Ross 508) sono stati suddivisi in 8 gruppi omogenei, allevati a terra su lettiera di torba. Quattro gruppi sono stati alimentati con una di dieta controllo contenente olio di oliva quale fonte lipidica, mentre gli altri quattro gruppi ricevevano una dieta in cui il supplemento lipidico era costituito da una miscela di olio al CLA in olio di oliva. Gli animali che hanno ricevuto la dieta trattata pur non differendo dal controllo per l'indice di conversione (1,90 vs 1,91), sono accresciuti meno (2,544 kg vs 2,639 kg, $P \leq 0,05$), producendo carcasse più magre con una minor quantità di grasso addominale (28,20 g vs 38,80 g, $P \leq 0,05$) e con una resa di macellazione più elevata (86,17% vs 85,66%, $P \leq 0,05$). Il profilo in acidi grassi della carne ha messo in evidenza un maggior contenuto in CLA ed un minor contenuto in OA per i soggetti trattati rispetto al controllo. Pertanto, è auspicabile l'integrazione delle diete per broiler con CLA ed OA ai fini di migliorare la qualità della carne in termini nutraceutici.

Parole chiave: Broilers, CLA, Acido Oleico, Qualità della carne.

Introduction

Several studies have clearly demonstrated that dietary Conjugated Linoleic Acid isomers (CLA) have an influence on body fat deposition (Banni and Martin, 1998; Dugan *et al.*, 1999), also modifying the fatty acids profile (Antongiovanni *et al.*, 2003).

The meat of ruminants contains higher amounts of CLA than that of non ruminant animals due to metabolism pathways occurring in the rumen (biohydrogenation) and in the adipose tissue. In fact, the highest CLA concentrations have been recorded in lamb and beef meat, whereas in pork and chicken meat CLA is usually lower than 1 mg/g lipids (Schmid *et al.*, 2006). Dugan *et al.* (1997) reported that CLA in the diet of growing pigs reduced feed intake and, consequently, decreased weight gains and subcutaneous fat deposition, with better feed/gain ratio. Similar results, with the exception of feed efficiency ratio, which was not improved, have been reported by Szymczyk *et al.* (2001), with broiler chickens.

Nutritional studies by Banni *et al.* (1996) and Kramer *et al.* (1998) demonstrated that dietary CLA is incorporated into membrane

phospholipids and that the accumulation of CLA in animal tissues depends upon the amount of CLA in the diet and total CLA intake (Ip *et al.*, 1994; Banni *et al.*, 1995).

The scientific interest in CLA has been increasing during the past decade as a result of its recognized beneficial effects on human health: anti-carcinogenic (Schultz *et al.*, 1992; Ip, 1997), hypocholesterolemic and anti-atherogenic properties (Lee *et al.*, 1994; Knekt *et al.*, 1996; Pariza *et al.*, 1996; Nicolosi *et al.*, 1997; Cesano *et al.*, 1998).

Similar properties may be ascribed to oleic acid (OA), the major fatty acid of olive oil (Frost Larsen *et al.*, 1999; Kris-Etherton *et al.*, 1999; Allman-Farinelli *et al.*, 2005; Nelson, 2005).

Szymczyk *et al.* (2001) found that the inclusion of CLA in the diets of broilers induced a decrease of OA in meat, with a significant linear response ($P \leq 0.01$) to dietary CLA. These results were confirmed by Thiel *et al.* (1998) with pigs and by Du and Ahn (2002) with broilers.

Since it appears desirable to produce CLA and OA naturally enriched meats for human consumption because of their better nutraceutical quality (Kalra, 2003), the

present study was designed and carried out in order to study the contemporaneous transfer of dietary CLA and OA into the meat of broilers and to monitor the live growth performance and carcass traits of the birds.

Material and methods

Birds and dietary treatments

Two hundred and forty Ross 308 one day-old female broiler chicks were randomly allotted to 2 dietary treatments: a basal diet supplemented with olive oil, in which the most important fatty acid was OA, as an energy source (control diet); and the same basal diet, but supplemented with olive oil enriched with 1% of a blend of CLA isomers (CLA diet), complementarily prepared by the company Silo, Florence (Italy). The fatty acids composition of olive oil and of the CLA supplement are shown in Table 1. Actually the birds were divided into 8 groups, kept

in 8 separate pens on a peat litter; 4 groups were fed the plain oil diets (control diet) and the other 4 groups were fed the CLA enriched oil ones (the treated animals). The birds were given free access to water and feed. The poultry house environment was controlled with a light regimen of 18 h light and 6 h dark. Three diets were used to meet the birds' requirements at different growing stages (according to NRC, 1994): a starter (0-7 d); a grower (8-21 d) and a finisher (22-47 d). The ingredient composition and the major nutritional traits of the 2 diets are reported in Tables 2 and 3. Each bird was individually identifiable by a leg ring, which was changed as the bird grew up.

On days 0, 8, 22 and 47 body weights were recorded individually, whereas feed intakes were recorded per pen.

At the end of the trial, on day 47, all the birds were slaughtered, plucked, eviscerated and the carcasses weighed. Then the carcasses were dissected into commercial

Table 1. Fatty acids of olive oil and of the CLA supplement (g/100 g fatty acids).

Fatty acid	Olive oil	CLA supplement
C _{14:0}	-	0.35
C _{16:0}	11.08	9.92
C _{16:1} n-7	-	0.25
C _{18:0}	2.82	4.14
C _{18:1} cis 9	70.82	30.52
C _{18:2} cis 9 cis 12	11.17	1.47
CLA cis 8 trans 10	-	4.00
CLA cis 9 trans 11	-	17.30
CLA trans 10 cis 12	-	20.00
CLA trans 11 cis 13	-	4.50
CLA cis cis	-	2.30
CLA trans trans	-	1.70
CLA other isomers	-	3.55
Other acids	4.11	-

Table 2. Ingredient composition (%).

Ingredient	Starter (0-7 d)	Grower (8-21 d)	Finisher (22-47 d)
Maize	52.05	57.00	58.00
Soy bean meal	35.50	33.10	31.05
Olive oil	4.50	5.25	6.30
Maize gluten feed	3.00	-	-
Dicalcium phosphate	1.90	1.90	1.90
Calcium carbonate	1.50	1.20	1.20
Sodium bicarbonate	0.25	0.25	0.25
Sodium chloride	0.25	0.25	0.25
DL Methionine	0.25	0.25	0.25
Lysine HCl	0.15	0.15	0.15
Choline chloride	0.15	0.15	0.15
Vitamin mineral premix	0.50	0.50	0.50
CLA blend supplement*	1.00	1.00	1.00

*The CLA supplement was present in the diets of treated animals only. In this case the amount of olive oil was 3.50, 4.25 and 5.30 kg/100 kg, respectively, for the three diets.

Table 3. Nutritional traits of diets.

		Starter (0-7 d)	Grower (8-21 d)	Finisher (22-47 d)
Dry matter	g/kg	895	863	879
Crude protein	"	245	213	206
Ether extract	"	72	91	95
Crude fibre	"	51	53	55
Ash	"	73	72	69
Calcium	"	12	11	11
Phosphorus	"	7	7	7
Lysine*	g/kg	12	11	10
Methionine*	"	4	3	3
Metabolizable Energy*	kcal/kg	2950	3010	3090

*Estimated from feed Tables (Sauvant *et al.*, 2004).

cuts according to Romboli *et al.* (1996). All the abdominal fat, including the adipose tissue lining the proventriculus and gizzard, was weighed as well. The skin thickness

was recorded by means of manual non-digital callipers.

The breasts of 6 birds per pen, taken at random, were analysed for total lipids and

fatty acids profiles. All samples were stored at -40°C prior to the analyses. In order to avoid variations in the cutting procedure, the same operator was employed every time.

Feeds and fatty acids analyses

Feed samples were analysed for dry matter, crude protein, crude fibre, ether extract and ash according to AOAC. (1990) method 954.01, 954.05 and 920.39 respectively. The procedures of extraction and methylation, preliminary to the analyses for the fatty acid profile, were different for the lipid supplements and meat samples, due to the different nature of matrixes. The oily supplements samples were dissolved in hexane and then the glycerides were methylated with sodium methylate in methanol ($\text{MeO}^-\text{Na}^+/\text{MeOH}$, 0.5 M), according to Christie (1982) and the free fatty acids component was methylated with diazomethane (Fales *et al.*, 1973). The meat samples consisting of 25 g of the homogenized whole breast, were extracted with chloroform-methanol (Folch *et al.*, 1957), evaporated under nitrogen and stored overnight on silica gel under vacuum (Lin, 2000; Roach *et al.*, 2002). Methyl esters were then prepared by the cited trans esterification of glycerides and phospholipids according to Christie (1982). In any case, nonadecanoic acid ($\text{C}_{19:0}$) was introduced as internal standard for the gas chromatographic analyses.

Methyl esters of medium and long chain fatty acids were analyzed by means of a gas-chromatograph apparatus (Dani, Milan, Italy) equipped with a FID detector and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands: 50×0.25 mm i.d.; film thickness 0.20 μm).

The column temperature programming was 120°C for 1 min, then up to 180°C by $5^{\circ}\text{C}/\text{min}$, held for 18 min, then up to 200°C by $2^{\circ}\text{C}/\text{min}$, held for 1 min, finally up to

230°C , again by $2^{\circ}\text{C}/\text{min}$. The injector and detector temperatures were 270°C and 300°C , respectively. Helium was the carrier gas with a flux of 1 ml/min and a split ratio of 1/100 (Sehat *et al.*, 1998a; Kramer *et al.*, 1999). The determination of C18:1 isomers and of total isomers of CLA was performed on a second aliquot of the same sample at 175°C (isothermal step) for 65 min, using a longer capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 100×0.25 mm i.d.; film thickness 0.20 μm). The identification of C18:1 and CLA isomers was based on a commercial standard mixture and published isomeric profiles (Wolf and Bayard, 1995). Since all methods using peak normalization and expressing results in relative percentage of the area of analyzed peaks are subjected to an over-evaluation because the areas of small peaks are not considered, the problem was avoided using nonadecanoic acid as the internal standard. All the results concerning fatty acid composition are expressed as g/100g of fat.

Geometrical and positional isomers of CLA were separated and identified by silver ion HPLC (Ha *et al.*, 1989; Sehat *et al.*, 1998a, 1998b; Eulitz *et al.*, 1999; Sehat *et al.*, 1999; Christie, 2001). The stationary phase was a silver ion column (ChromSpher lipid column, 4.6 mm i.d. x 250 mm stainless steel, 5 μ particle size, commercially available in the form of silver ion). The mobile phase was a fresh mixture of acetonitril in hexane (0.1% v/v). The injection loop was 10 μl . The solvent flow rate was standardized at 1 ml/min and UV was set at 233 nm. Since a reliable internal standard for CLA is not yet available, the quantitative measurements were performed through a calibration curve (Mattreya Inc. 500 Tressler St. Pleasant Gap, PA and Sigma Aldrich) and data were referred to gas-chromatographic results.

The desaturation index was calculated according to Mele *et al.* (2007).

Statistical analysis

All data referable to the single different treatments were analysed by means of the Statistical Analysis System (SAS, 1999) by one-way ANOVA, keeping the factor "diet" as the fixed one.

Results and discussion

Feed intakes resulted statistically not different between the control birds and the CLA treated ones during the first two growth periods (Table 4) and there was no mortality. The feed intake of treated birds resulted lower all the time; the difference became significant ($P \leq 0.05$) in the finishing phase only. The same behaviour was observed for the whole period (0-47 d), characterized by

a statistically significant ($P \leq 0.05$) feed intake depression for birds fed the CLA diet (from 4.99 kg to 4.88 kg). The weight gains of treated birds were lower ($P \leq 0.01$ in the first week and $P \leq 0.05$ in the finishing period), but higher in the mid phase ($P \leq 0.05$). The final result was that the treated chickens ate less feed (about 115 g in the whole period, $P \leq 0.05$), had a slightly lighter final weight (about 95 g less, $P \leq 0.05$) and, consequently, the feed intake/gain efficiency ratios were comparable if referred to the whole growth period.

Some differences in carcass weight (after feathers and gut have been removed) between CLA and control diets were found in our experiment. The results are shown in Table 5. The dressing out percentage in-

Table 4. Live performance of birds per single growth period.

		Control diet	CLA diet	SE
Starter (0-7 d)				
Weight gain	g	83.3 ^A	72.4 ^B	2.30
Feed intake	"	97.5	94.2	2.90
Feed/gain ratio		1.17 ^A	1.30 ^B	0.03
Grower (8-22 d)				
Weight gain	g	591.9 ^a	602.9 ^b	8.20
Feed intake	"	840.5	838.0	3.00
Feed/gain ratio		1.42 ^a	1.39 ^b	0.02
Finisher (23-47 d)				
Weight gain	g	1,924.7 ^a	1,829.2 ^b	40.40
Feed intake	"	4,061.1 ^a	3,951.0 ^b	28.91
Feed/gain ratio		2.11 ^a	2.16 ^b	0.04
Whole period (0-47 d)				
Body weight	g	2,639.9 ^a	2,544.5 ^b	29.61
Weight gain	"	2,599.0 ^a	2,504.5 ^b	29.69
Feed intake	"	4,999.1 ^a	4,883.2 ^b	28.91
Feed/gain ratio		1.89	1.92	0.01

^{A, B} = $P \leq 0.01$; ^{a, b} = $P \leq 0.05$.

Table 5. Major carcass traits of slaughtered birds.

		Control diet	CLA diet	SE
Dressing out	%	85.66 ^a	86.17 ^b	0.05
Breast	g	492.67 ^a	468.67 ^b	0.80
Leg	"	513.67 ^a	495.00 ^b	1.20
Breast skin thickness	mm	0.55 ^a	0.48 ^b	0.01
Leg skin thickness	"	1.57 ^a	0.98 ^b	0.03
Drumstick thickness	"	0.47 ^a	0.41 ^b	0.01
Liver	g	54.67	54.67	0.50
Abdominal fat	"	38.80 ^a	28.20 ^b	0.50

^{a, b}= $P \leq 0.05$.

creased in CLA treated animals ($P \leq 0.05$), while abdominal separable fat was significantly reduced ($P \leq 0.05$). The skin thickness of breast and drumstick decreased with CLA treatment and all the other parts of the carcass were lighter in the treated birds, with the only exception of the liver, as reported in Table 5. However, the percentage value of each carcass trait respect to the animal body weight did not show differences between the two groups. In fact, the parts of the carcass from treated animals belong to animals with a lower weight. So, the ratio single carcass trait/body weight was not characterized by significant differences (breast 18.66% vs 18.42%, leg 19.45% vs 19.46%, breast skin thickness 0.02% vs 0.02, leg skin thickness 0.06% vs 0.04%, drumstick skin thickness 0.02% vs 0.02%, liver 2.07% vs 2.15%) with the exception of abdominal fat (1.47% vs 1.11%, $P \leq 0.05$).

The lipid concentration of breast meat was low and similar between the groups, ranging from 1.62 g/100g of meat for the CLA diet to 1.65 g/100g of meat for the control one (Table 6). On the contrary, the fatty acid profile resulted affected by dietary CLA not only quantitatively, but also qualitatively, as shown in Table 6. Linoleic (LA; C_{18:2} cis 9 cis

12), linolenic acid (LNA, C_{18:3} n-3), OA (C_{18:1} cis 9), C_{18:1} cis 11 and arachidonic acid (AA; C_{20:4} n-6) were significantly ($P \leq 0.05$) depressed in the CLA treated birds, although the OA percentage remained high in both groups because the two dietary lipid supplements contained olive oil. No trans C_{18:1} isomers were found in appreciable concentration. Myristic (C_{14:0}) and palmitic (C_{16:0}) acids resulted increased, and this does not signify an improvement in the meat quality (Ulbricht *et al.*, 1991). On the contrary, the percentage of stearic acid was significantly decreased when the CLA supplement was included in the diet. The meat of the animals fed the control diet did not contain CLA isomers while the fatty acids profile of meat from treated birds was characterised by the appreciable presence of only the isomers trans10, cis12 CLA and cis9, trans11 CLA, as shown in Table 6.

The results of the *in vivo* performances do not appear in agreement with those of Szymczyk *et al.* (2001) and Sirri *et al.* (2003). The former authors showed that the main effect of dietary CLA occurs during the period 8-22 days with a decrease in body weight gain and the same feed conversion efficiency. On the contrary, Sirri *et al.* (2003) in their

Table 6. Fatty acid composition of breast meat (g/100 g fat).

Fatty acid	Control diet	CLA diet	SE
C14:0	0.70 ^a	0.97 ^b	0.10
C16:0	24.69 ^a	30.40 ^b	2.00
C16:1 n-7	2.70	2.96	0.59
C18:0	8.46 ^a	6.34 ^b	0.45
C18:1 cis 9	34.26 ^a	28.95 ^b	2.08
C18:1 cis 11	2.54 ^a	1.73 ^b	0.16
C18:2 cis 9 cis 12	10.12 ^a	8.20 ^b	0.49
C18:3 n-3	0.40 ^a	0.17 ^b	0.10
CLA cis 9 trans 11	-	0.36	0.06
CLA trans 10 cis 12	-	0.26	0.03
CLA other isomers	-	0.25	0.09
C20:4 n-6	1.22 ^a	0.37 ^b	0.28
Lipid concentration (g/100g meat)	1.65	1.62	0.03
Desaturation index	0.52 ^a	0.46 ^b	0.09

^{a, b}= $P \leq 0.05$.

work showed that daily weight gain and feed intake were not affected by the CLA treatment of birds.

The increase in dressing out percentage in CLA treated animals ($P \leq 0.05$) was related to the significant decrease in abdominal separable fat ($P \leq 0.05$). These effects could be attributed to the ability of CLA to reduce body fat accretion. Park *et al.* (1997) and Ostrowska *et al.* (1999) reported that in rats and in pigs CLA is able to reduce lipoprotein lipase activity and to increase lipolysis in cultured murine adipocytes; in fact, CLA seems to lead to a reduction in fat deposition, altering body composition. Moreover, the trans 10 isomers seem to be principally responsible for this action (Griinari *et al.*, 1998).

Examining the fatty acids profile, it resulted affected by dietary CLA not only quantitatively, but also qualitatively. The increase in myristic, palmitic and stearic

acid was in agreement with some results reported in literature. In fact, similar data have been reported by Sirri *et al.* (2003) and by Du *et al.* (2002) who explained this trend with the inhibition of the liver Δ^9 desaturase, caused by the presence of CLA in tissues in which this enzyme is active (Lee *et al.*, 1995, 1998). This seems to be confirmed by a higher value of desaturation index (Table 6) for the control diet than the CLA diet (Malau-Aduli *et al.*, 1997). The increase in stearic acid percentage could be considered positively because this fatty acid is a precursor of OA in tissues and it may be desaturated by Stearoyl-CoA (Ulbricht *et al.*, 1991). The lower content of LA, precursor of AA in the linoleic fall down to AA, in treated meats could have induced a lower conversion of this fatty acid to AA, as it seems to have occurred in the present experiment. Moreover, Belury and Kempa-Stecczo (1997) proposed the hypothesis

that CLA, acting as a substrate for Δ^6 desaturase, could inhibit the desaturation of linoleic acid to AA with the consequent synthesis of conjugated AA; in fact, the percentage of this fatty acid decreased in meats of treated animals. The dramatic depression of arachidonic acid is to be considered an important achievement in terms of beneficial effects on the health of consumers, since arachidonic acid is a precursor to some proinflammatory eicosanoids (British Nutrition foundation, 1992).

Since the lipid supplements of both diets did not contain Vaccenic acid, the precursor of cis 9, trans 11 CLA in tissues, the finding that no CLA isomers were found in the meat fat of control birds confirms the fact that chickens are not capable of synthesizing CLA for themselves but they need a precursor on which the Stearoyl-CoA desaturates (Lefevre *et al.*, 1999) may operate. The CLA isomers detected in the meat fat of treated birds came from the supplemented feed and it is clear that they have been absorbed and incorporated preferentially: the trans 10 cis 12 isomer was higher than the cis 9 trans 11 in the supplement (Table 1), but was much lower in the meat fat (Table 6); cis 8 trans 10 was present in the supplement, but was not found in the meat lipids. So, the preferential incorporation of individual isomers into body lipids was confirmed. Szymczyk *et al.* (2001), who reported that the fatty acid profile of the lipid fraction of broiler meat does not reflect the composition of commercial CLA supplements, explained this effect with a lower efficiency of trans 8, cis 10 and trans 10,

cis 12 CLA to be incorporated into tissue lipids.

Conclusions

The results of the present feeding trial confirm that it is possible to improve the quality of poultry meat by supplementing feeds with a commercial blend of CLA isomers. Our experiment demonstrated that a CLA addition to broiler diet affected the performances of birds, inducing leaner carcasses. By including the CLA and OA supplements in animal diets, it was possible to improve the percentage of these healthy fatty acids and, contemporarily, to reduce the content of AA in meat with the aim of producing a food with a higher nutritional value for the consumer. Moreover, including olive oil as a supplement in broiler diets, it is possible to increase the OA percentage through the presence of a high concentration of CLA in the diet.

In conclusion, the supplementation of broiler feeds with a commercial blend of CLA isomers could be recommended to produce leaner carcasses and to modify the fatty acid profile of meat. Further efforts should be made to enrich the blend with the cis 9 trans 11 isomer, the most beneficial one, as much as possible.

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