

The effects of dietary conjugated linoleic acid (CLA) on fatty acid composition and key enzymes of fatty acid oxidation in liver and muscle of geese

Xuhui ZHANG, Baowei WANG*, Fangyu LONG, Lei WANG, Zhigang YANG

Waterfowl Research Institute, Qingdao Agricultural University, Shandong, 266109, P.R. CHINA

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Abstract: The objective of this study was to determine the influence of conjugated linoleic acid (CLA) on fatty acid composition and key enzymes of fatty acid oxidation in liver and muscle of geese. A total of 192 1-day-old geese were randomly assigned to 1 of 4 dietary groups, and were fed for 56 days on diets containing 2.5% soybean oil (group A, control group) or 2.0% soybean oil with 0.5% CLA (group B), 1.0% soybean oil with 1.5% CLA (group C), and 2.5 % CLA (group D). Geese fed CLA had a decreased abdominal fat percentage (AFP) ($P < 0.01$) compared with the control. It was observed that dietary CLA levels had a significant effect on the body weight, weight gain, feed conversion rate (FCR) ($P < 0.05$), and feed intake ($P < 0.01$). Fatty acid composition showed a significant increase of the biologically active cis-9, trans-11 and trans-10, and cis-12 CLA isomers in both liver and muscle of geese fed CLA ($P < 0.01$). Dietary CLA led to an increase ($P < 0.01$) in saturated fatty acid (SFA) and a reduction in monounsaturated fatty acid (MUFA) ($P < 0.05$) concentrations in both liver and muscle. In liver, dietary CLA increased ($P < 0.05$) acyl CoA oxidase (ACO) activity, but did not affect carnitine palmitoyltransferase-I (CPT-I) activity compared with the control. CPT-I activity was significantly increased by 2.5% dietary CLA in muscle, where ACO activity was decreased at 1.5% CLA level. The results obtained suggested that geese can successfully incorporate CLA in both liver and muscle, which could be beneficial in the human diet, through provision of bioactive fatty acids with no detrimental effects on n-3 PUFA levels.

Key Words: Conjugated linoleic acid, CLA, fatty acid oxidation, key enzymes, geese

Introduction

Conjugated linoleic acid (CLA) is a term used to describe positional and geometric isomers of linoleic acid (18:2n-6; LA), the 2 main naturally occurring isomers being cis-9, trans-11 and trans-10, and cis-12. These compounds occur particularly in dairy products and ruminant meats, such as beef and lamb, but are widespread at lower levels in many foodstuffs (1,2).

Dietary inclusion of CLA can cause significant alterations in energy and lipid metabolism in mammals leading to reductions in overall body fat mass. This has been suggested to be a positive effect in a variety of farmed species and animal disease models and by extension, humans (3-5). The exact mechanism for the reduced fat accumulation by dietary CLA is not clear, one of which is owing to the promotion of fatty acid oxidation (6).

* E-mail: wangbw@qau.edu.cn

Dietary CLAs were shown to change the fatty acid composition of animal tissues in the studies conducted on Japanese quail and pigeon (7,8). Therefore, a further increase in its CLA content could be of interest to enhance the nutritional value and decrease the fat deposition of geese for human consumption. In light of the above, the aim of the present study is to investigate the effects of graded dietary levels of CLA on lipid metabolism, fatty acid compositions of liver and muscle, and key enzymes of fatty acid oxidation in geese, with an expectation to increase the value and benefit of the corresponding geese product for human consumption from the potential use of CLA.

Materials and Methods

Preparation of the diet

Geese were fed corn-soybean based diets and supplemented with 0% CLA (containing 2.5% soybean oil, group A, control), 0.5% CLA (containing 2.0% soybean oil, group B), 1.5% CLA (containing 1.0% soybean oil, group C), 2.5% CLA (containing 0.0% soybean oil, group D). The ingredients of the diet were bought from a local market (Qingdao Shandong, China), and diet preparation was carried out at the experimental animal house, Qingdao Agricultural University, Shandong, China before the feeding trial. The geese were fed a starter diet until 28-day-of age followed by a grower diet from 29 to 56 days, and the diets were formulated to satisfy the nutritional requirements of geese (P.R. the Act of Technical Specification for Wulong-geese Production; Serial Number: DB37/T503-2004, 2004), and each diet was formulated to be isoenergetic, isolipidic, and isonitrogenous (Table 1). The oils used during diet preparation were soybean oil, either alone or in combination with CLA (containing 80% CLA free fatty acid with a mixture of 36.7% cis-9, trans-11 and 39.5% trans-10, cis-12 isomers) and purchased from Qingdao Aohai Biologic Limited Company, Shandong, China. CLA was added to the basal diet at the expense of soybean oil. Moreover, BHT was added to minimize the oxidation of the fatty acids.

Animals and experimental design

The experiment was carried out at the experimental animal house, Qingdao Agricultural

University, Shandong, China, and lasted 56 days. One hundred and ninety two 1-day-old geese with an average initial body weight of 79 ± 1.13 g (mean \pm S.D.) were selected and randomly assigned into 1 of 4 dietary treatments with 4 replicates per treatment according to a completely randomized design. Then, geese in each treatment were fed the prepared diet containing 0.0% CLA (group A, the control), 0.5% CLA (group B), 1.5% CLA (group C), and 2.5% CLA (group D). Trial geese received immunoprophylaxis, and they had free access to diet and water. Data on feed intake and body weight of geese were collected weekly and mortality in the groups was recorded daily. All experimental procedures were performed according to the Guide for Animal Care and Use of Laboratory Animals in the Institutional Animal Care and Use Committee of Qingdao Agricultural University. The experimental protocol was approved by the Department Animal Ethics Committee of Qingdao Agricultural University.

Samples collected

At the end of the feeding trial, geese were deprived of food for 12 h. Three geese were randomly selected from each replicate; after weighed, all geese were killed by exsanguinations and slaughtered immediately. The samples of liver, leg, and breast muscle were removed and immediately frozen in liquid nitrogen and stored in a freezer at -80 °C for analysis. Then, the abdominal fat was collected and weighed.

Analyses of the fatty acid composition

Leg and breast muscle samples from the same geese were homogenized into pooled "pates". Total lipid was prepared according to the method of Folch et al. (9). The weight of lipid was determined gravimetrically after evaporation of solvent and overnight vacuum dry as described previously (10). Fatty acid methyl esters (FAME) from diets and tissue total lipid were prepared by acid-catalyzed transesterification of total lipid similar to the method of Tocher and Harvie (11) except that the reaction was performed at 80 °C for 3 h. FAME were prepared by reaction with 4% HCl in methanol for 20 min at 60 °C. FAME were separated and quantified by gas-liquid chromatography (Carlo Erba Vega 8160, Milan, Italy) using a 30 m \times 0.32 mm i.d. capillary column (CP Wax 52CB, Chrompak, London, U.K.) and on-

Table 1. Chemical composition of the diets commercially prepared (as fed basis).

	0-28 days				29-56 days			
	Dietary treatments ²							
	A	B	C	D	A	B	C	D
Formulation (%)								
Maize	59.0	59.0	59.0	59.0	54.9	54.9	54.9	54.9
Soya bean meal	30.5	30.5	30.5	30.5	24.0	24.0	24.0	24.0
Chinese wild rye meal	4.5	4.5	4.5	4.5	15.0	15.0	15.0	15.0
Soya bean oil	2.5	2.0	1.0	0	2.5	2.0	1.0	0
CLA	0	0.5	1.5	2.5	0	0.5	1.5	2.5
Limestone	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
CaHPO ₄	1.32	1.32	1.32	1.32	1.40	1.40	1.40	1.40
NaCl	0.30	0.30	0.30	0.30	0.35	0.35	0.35	0.35
Premix ¹	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DL-Methionine (g/kg)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Lysine (g/kg)	0	0	0	0	1.5	1.5	1.5	1.5
Chemical composition								
Dry matter, g/kg	900	900	900	900	900	900	900	900
Metabolisable energy, MJ/kg	11.74	11.74	11.74	11.74	10.94	10.94	10.94	10.94
Crude protein, g/kg	190.0	190.0	190.0	190.0	165.0	165.0	165.0	165.0
NaCl, g/kg	3.0	3.0	3.0	3.0	3.5	3.5	3.5	3.5
Crude fiber, g/kg	42	42	42	42	65	65	65	65
Calcium, g/kg	8	8	8	8	8	8	8	8
Available phosphorus, g/kg	4	4	4	4	4	4	4	4
Lysine, g/kg	9.54	9.54	9.54	9.54	9.08	9.08	9.08	9.08
Methionine+Cystine, g/kg	7.43	7.43	7.43	7.43	6.88	6.88	6.88	6.88

¹ Contained per kg of diet: Vitamin A, 1500 IU; Vitamin D, 200 IU; Vitamin E, 12.5 mg; Vitamin K, 1.5 mg; Vitamin B₁, 2.2 mg; Vitamin B₂, 5.0 mg; Vitamin B₆, 2 mg; Niacin, 65 mg; D-pantothenic acid, 15 mg; Biotin, 0.2 mg; Folic acid, 0.5 mg; Sinkaline, 500 mg; Fe, 96 mg; Cu, 5 mg; Mn, 66 mg; Zn, 60 mg; I, 0.42 mg; Se, 0.15 mg

² Dietary treatments: Group A-D, dietary CLA at an inclusion level of 0.0%, 0.5%, 1.5%, and 2.5%, respectively.

column injection. Hydrogen was used as carrier gas and temperature programming was from 50 °C to 150 °C at 40 °C min⁻¹ and then to 230 °C at 2.0 °C min⁻¹. Methyl esters were identified and quantified as described previously (10). Individual fatty acid methyl esters were expressed as percentage of all peaks.

Analyses of key enzymes of fatty acid oxidation

The carnitine palmitoyltransferase-I (CPT1) activity in the selected tissues was determined from the tissue homogenates using a slight modification of the method of Kim et al. (12) developed by Zierz and Engel (13). The enzymatic reaction was initiated by adding palmitoyl-CoA (100 µM) and carnitine (400 µM) to generate palmitoylcarnitine, and the mixture was incubated at 37

°C for 3 min. CPT-1 activity, determined by the initial rate of decrease in palmitoyl-CoA, was assessed using spectrophotometry at 324 nm according to Hamdan et al. (14). AcylCoA oxidase (ACO) activity was measured by a spectrophotometric assay based on the determination of hydrogen peroxide production coupled to the oxidation of leucodichlorofluorescein (DCF) in a reaction catalyzed by exogenous peroxidase (15).

Statistical analysis

All data are presented as means ± S.D. The data were analyzed by analysis of variance for repeated measures using the general linear models procedure of SAS (Version 6.12). Significance was evaluated at the 0.05 level.

Results

The effects of dietary supplementation of CLA on body weight, weight gain, feed intake, and FCR are presented in Table 2. Differences were significant ($P < 0.05$) between Group A and Group D, and not significant ($P > 0.05$) between Group A (the control), Group B, and Group C with respect to body weight and weight gain during the 2 growth phases of 0-28 and 29-56 days. Geese fed CLA had lower ($P < 0.01$) levels of AFP than the control. Feed intake for group D was significantly ($P < 0.01$) lower than the other groups.

The fatty acid compositions in both the liver and muscle are shown in Tables 3 and 4. Dietary CLA resulted in the deposition into liver and muscle lipids, and it showed a significant increase of the biologically active cis-9, trans-11 and trans-10, cis-12 CLA isomers in CLA-fed geese than not given CLA ($P < 0.01$), but it was noteworthy that CLA was deposited to a greater extent in liver lipids than in muscle, reaching 2.82% and 1.31% of total fatty acids, respectively, in geese fed 2.5% CLA.

Dietary CLA increased ($P < 0.01$) saturated fatty acid (SFA) content, and decreased ($P < 0.05$) monounsaturated fatty acid (MUFA) content in both liver and muscle, also the total polyunsaturated fatty acids (PUFA) content decreased ($P < 0.05$) in muscle.

The palmitoleic acid, oleic acid content, linoleic acid, and linolenic acid tended to be lower ($P < 0.05$) in CLA-fed geese than in geese not given CLA, and myristic acid, palmitic acid, and stearic acid presented to be higher ($P < 0.05$).

The results of CPT-I and ACO activities are shown in Figure 1 and 2. No significant effects ($P > 0.05$) on CPT-I activities were observed in liver of geese, where ACO activities were significantly increased ($P < 0.05$) by any dietary CLA treatment. In muscle, CPT-I activities were significantly increased ($P < 0.05$) by the dietary level of 2.5% CLA, although it was only by the dietary level of 2.5% CLA. There was a significant decrease ($P < 0.05$) in ACO activities between 1.5% CLA group and the control, and no significant effects ($P > 0.05$) were observed among the other groups.

Discussions

Szymczyk et al. (16) reported that there was a significant decrease (from 2.68% to 1.78%) in AFP with different levels of CLA (0.0%, 0.5%, 1.0%, or 1.5%) in chicken diet. Sisk et al. (17) reported that there was a dose-dependent effect of CLA in the regulation of body fat tissue in rats, so there would be a hindrance in the metabolism of fat and a reduction

Table 2. Effects of dietary CLA treatments on growth performance of geese.

	Dietary treatments			
	Group A	Group B	Group C	Group D
0-28 days				
Body weight, g	1143 ± 65.57 ^a	1204 ± 74.56 ^a	1201 ± 71.55 ^a	795 ± 57.27 ^b
Weight gain, g	1064 ± 66.36 ^a	1126 ± 74.16 ^a	1122 ± 71.98 ^a	715 ± 56.33 ^b
Feed intake, g	2580 ± 161.59 ^a	2369 ± 156.71 ^b	2374 ± 152.43 ^b	1485 ± 117.18 ^c
FCR	2.43 ± 0.01 ^a	2.10 ± 0.02 ^b	2.12 ± 0.02 ^b	2.08 ± 0.01 ^b
AFP (%)	0.53 ± 0.05 ^a	0.48 ± 0.06 ^a	0.37 ± 0.05 ^b	0.09 ± 0.07 ^c
29-56 days				
Body weight, g	2719 ± 156.23 ^a	2947 ± 189.36 ^a	2913 ± 173.90 ^a	1763 ± 142.17 ^b
Weight gain, g	1624 ± 138.70 ^a	1787 ± 169.18 ^a	1792 ± 152.07 ^a	968 ± 120.94 ^b
Feed intake, g	6824 ± 202.54 ^a	5806 ± 226.00 ^b	5697 ± 272.31 ^b	2941 ± 207.01 ^c
FCR	4.20 ± 0.01 ^a	3.28 ± 0.02 ^b	3.29 ± 0.01 ^b	3.04 ± 0.01 ^b
AFP (%)	1.57 ± 0.13 ^a	0.94 ± 0.15 ^b	0.84 ± 0.09 ^b	0.50 ± 0.06 ^c

^{a-c} Means within rows with different superscripts differ at $P < 0.01$ (**) and $P < 0.05$ (*); n = 12; FCR: feed conversion ratio (kg feed intake/kg weight gain); AFP: percentage of abdominal fat.

Table 3. Effects of dietary CLA treatments on fatty acid composition of liver in geese.

Fatty acids	Fatty acid (% of total FAEM)			
	Group A	Group B	Group C	Group D
C14:0	2.40 ± 0.53 ^b	2.42 ± 0.23 ^b	3.32 ± 0.20 ^a	3.89 ± 0.22 ^a
C16:0	22.82 ± 1.54 ^b	23.73 ± 1.68 ^{ab}	25.38 ± 2.09 ^a	25.18 ± 1.91 ^a
C16:1, n-7	3.14 ± 0.65	4.18 ± 0.79	3.40 ± 0.46	3.92 ± 0.56
C18:0	5.64 ± 0.56 ^c	6.82 ± 0.75 ^b	7.23 ± 0.69 ^b	9.38 ± 0.76 ^a
C18:1, n-9	42.40 ± 3.21 ^a	41.90 ± 2.54 ^a	38.18 ± 2.95 ^b	35.52 ± 2.84 ^b
C18:2, n-6	17.62 ± 2.92 ^a	15.38 ± 3.29 ^b	15.91 ± 2.73 ^b	13.63 ± 1.56 ^c
cis-9, trans-11 CLA	ND	0.37 ± 0.05 ^c	0.81 ± 0.04 ^b	1.57 ± 0.07 ^a
trans-10, cis-12 CLA	ND	0.32 ± 0.02 ^c	0.65 ± 0.03 ^b	1.24 ± 0.09 ^a
C18:3, n-3	1.02 ± 0.13 ^a	0.63 ± 0.15 ^b	0.61 ± 0.14 ^b	0.50 ± 0.10 ^b
C20:4, n-6	0.53 ± 0.12	0.45 ± 0.07	0.54 ± 0.09	0.49 ± 0.04
ΣCLA	ND	0.69 ± 0.05 ^c	1.46 ± 0.42 ^b	2.82 ± 0.51 ^a
ΣSFA	30.86 ± 3.49 ^c	32.97 ± 2.35 ^c	35.93 ± 3.28 ^b	38.45 ± 3.78 ^a
ΣMUFA	45.54 ± 4.29 ^a	46.08 ± 3.97 ^a	41.58 ± 4.02 ^b	39.44 ± 4.18 ^c
ΣPUFA	19.17 ± 1.24	17.15 ± 1.65	18.52 ± 1.89	17.43 ± 1.94
UP	4.43 ± 0.75	3.80 ± 0.77	3.97 ± 0.71	4.68 ± 0.44

^{a-c} Means within rows with different superscripts differ at P < 0.01 (**) and P < 0.05 (*); n = 12; ΣSFA: total saturated fatty acids; ΣMUFA: total monounsaturated fatty acids; ΣPUFA: total polyunsaturated fatty acids; ΣCLA: total conjugated linoleic acids; UP: unidentified peaks; ND: not detectable.

Table 4. Effects of dietary CLA treatments on fatty acid composition of muscle in geese.

Fatty acid	Fatty acids (% of total FAEM)			
	Group A	Group B	Group C	Group D
C14:0	1.15 ± 0.18 ^b	1.51 ± 0.29 ^b	1.96 ± 0.21 ^a	2.23 ± 0.31 ^a
C16:0	19.23 ± 1.29 ^b	19.19 ± 1.32 ^b	20.40 ± 2.15 ^b	24.62 ± 1.38 ^a
C16:1, n-7	5.96 ± 0.98	5.61 ± 0.63	5.35 ± 0.50	5.02 ± 0.62
C18:0	4.89 ± 0.39 ^c	5.75 ± 0.53 ^c	8.69 ± 0.39 ^b	9.28 ± 0.32 ^a
C18:1, n-9	43.42 ± 2.47 ^a	43.57 ± 2.87 ^a	40.46 ± 3.05 ^{ab}	38.70 ± 3.92 ^b
C18:2, n-6	19.20 ± 2.38 ^a	17.34 ± 2.19 ^{ab}	17.47 ± 2.57 ^{ab}	15.67 ± 2.75 ^b
cis-9, trans-11 CLA	ND	0.09 ± 0.03 ^c	0.32 ± 0.01 ^b	0.71 ± 0.08 ^a
trans-10, cis-12 CLA	ND	0.13 ± 0.01 ^c	0.27 ± 0.03 ^b	0.60 ± 0.07 ^a
C18:3, n-3	1.12 ± 0.05 ^a	0.94 ± 0.04 ^b	0.83 ± 0.02 ^b	0.64 ± 0.06 ^c
C20:4, n-6	2.03 ± 0.49 ^b	1.67 ± 0.23 ^{ab}	1.26 ± 0.19 ^b	0.95 ± 0.38 ^c
ΣCLA	ND	0.22 ± 0.03 ^c	0.59 ± 0.45 ^b	1.31 ± 0.39 ^a
ΣSFA	25.27 ± 3.49 ^c	26.45 ± 2.35 ^c	31.05 ± 3.28 ^b	36.13 ± 3.78 ^a
ΣMUFA	49.38 ± 4.29 ^a	49.18 ± 3.97 ^a	45.81 ± 4.02 ^b	43.72 ± 4.18 ^b
ΣPUFA	22.35 ± 1.42 ^a	19.95 ± 1.65 ^{ab}	19.56 ± 1.89 ^{ab}	17.26 ± 1.94 ^b
UP	3.00 ± 0.56 ^{ab}	4.20 ± 0.78 ^a	2.99 ± 0.83 ^{ab}	1.58 ± 0.39 ^b

^{a-c} Means within rows with different superscripts differ at P < 0.01 (**) and P < 0.05 (*)

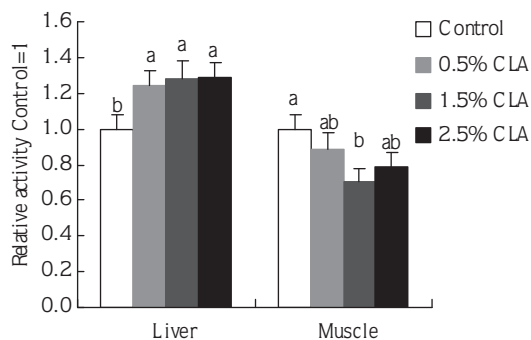


Figure 1. Effects of CLA on CPT-I activities in tissue homogenates of the liver and muscle of geese (n = 12).

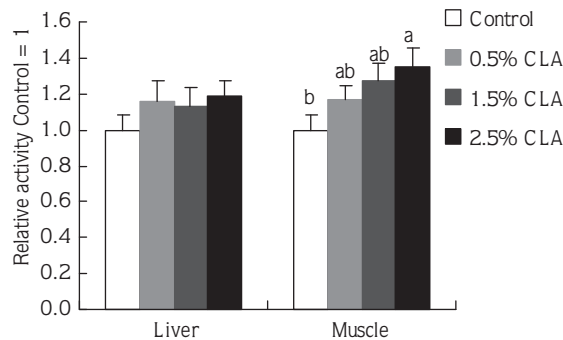


Figure 2. Effects of CLA on ACO activities in tissue homogenates of liver and muscle of geese (n = 12).

in body fat in rats if they were fed CLA over a long term (18). At the end of this trial, CLA of different levels reduced the AFP of geese from 1.57% to 0.5% ($P < 0.01$). It is obviously that CLA is especially important in the catabolism of abdominal fat. Proposed anti-obesity mechanisms of CLA include decreased preadipocyte differentiation and proliferation (19), decreased lipogenesis (20,21), and increased lipolysis and fatty acid oxidation (6).

The changes in fatty acid profile in relation to CLA supplementation are consistent with those observed in studies of CLA supplementation in pigs (22,23), quails (7), and pigeons (8). The lowered MUFA levels and increased in SFA levels in CLA-fed animals are probably due to inhibition of $\Delta 9$ -desaturase by CLA, since other in vivo and also in vitro studies have shown that CLA reduces $\Delta 9$ -desaturase activity either by suppressing mRNA expression (24) or reducing the activity of the enzyme (25) thereby reducing MUFA levels. Smith et al. (26) reported a lowered $\Delta 9$ -desaturase inside in CLA-fed pigs.

Studies have suggested that CLA increased fatty acid oxidation in mouse liver (27) and a variety of tissues in rats (28) via an increase in CPT-I activity. CPT-I is considered the rate-limiting enzyme that regulates fatty acid oxidation in mitochondria. This enzyme catalyzes the formation of acyl-carnitine from acyl-CoA. This reaction is the first step in the transport of long-chain fatty acids from the cytosol into the mitochondrial matrix for fatty acid oxidation (29). As such, it plays a central role in the partitioning of fatty acids between mitochondrial oxidation and their accumulation as long-chain acyl-CoA (LC-CoA) and/or complex lipids in the cytoplasm. The present study showed that CPT-

I activity in geese liver was not increased significantly ($P > 0.05$) by dietary CLA, suggesting that mitochondrial β -oxidation would not be increased. In contrast, ACO in liver was significantly increased ($P < 0.05$) by dietary CLA, suggesting that dietary CLA may have a greater effect on peroxisomal rather than mitochondrial fatty acid oxidation in geese liver. In muscle, CLA feeding appeared to enhance mitochondrial β -oxidation which was reflected in a significant increase ($P < 0.05$) in CPT-I activity by the higher level of dietary CLA (2.5%). Therefore, CPT-I activity and the concomitant transport of fatty acids as acyl-CoAs into the mitochondria are enhanced to produce energy by β -oxidation. In contrast, peroxisomal fatty acid oxidation was inhibited ($P < 0.05$) by 1.5 % dietary CLA, which showed an inhibition of long chain fatty acid from catalyzing by dehydrogenation in geese muscle.

In conclusion, we found that dietary supplementation of CLA to geese decreased body weight, weight gain, feed intake, FCR, and AFP. Furthermore, CLA supplementation modified the fatty acid composition. The deposition of biologically active cis-9, trans-11 CLA and trans-10, cis-12 CLA isomers in CLA-fed geese suggests that the nutritional quality for human consumption may be improved, contributing to the production of a functional food.

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