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Effects of conjugated linoleic acid on growth performance, feed conversion efficiency, and subsequent carcass quality in broiler chickens

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The effect of dietary conjugated linoleic acid isomers (CLA) on growth performance, carcass composition, fatty acid composition of adipose and muscle tissues, and serum lipoproteins was investigated in broiler chickens. A total of 160 (eighty male and eighty female) chickens were allocated to four dietary treatments (0·0, 0·5, 1·0, and 1·5 % CLA) and fed a standard starter diet from 8 to 21 d, and a grower-finisher diet from 22–42 d. When determined for the total period 8–42 d, feed intake and body weight gains of broiler chickens were significantly reduced (from 3·31 to 3·12 kg and from 1615 to 1435 g respectively; $P < 0·05$), particularly at the 1·5 % dietary CLA level. Feed conversion efficiency and carcass yield values showed no significant effects of dietary CLA. Abdominal fat deposition was significantly reduced (from 2·68 to 1·78 %; $P < 0·05$), the relative proportion of breast muscles was unaffected, and that of leg muscles significantly increased (from 19·0 to 20·6 %; $P < 0·05$). The concentration of CLA isomers (% of total methyl esters of fatty acids) increased linearly in tissue samples from broilers fed 0·5, 1·0, and 1·5 % dietary CLA. The relative proportions of saturated fatty acids (16:0, 18:0) were significantly ($P < 0·01$) increased, and those of monounsaturated (16:1, 18:1) and polyunsaturated fatty acids (18:2, 20:4 in muscle tissues) significantly ($P < 0·05$) reduced. Total serum cholesterol concentrations reached a maximum in broilers fed 1·0 % CLA and then decreased slightly (from 141·73 to 136·47 mg/dl; $P < 0·01$). The same was true also for HDL-cholesterol (from 113·58 to 109·97 mg/dl; $P < 0·01$). The HDL cholesterol:total cholesterol ratio and serum triacylglycerol concentration was unaffected. In conclusion, feeding CLA to broiler chickens resulted in substantial incorporation of CLA isomers into their tissue lipids, thus providing a potential CLA-rich source for human consumption.

Conjugated linoleic acid: Growth performance: Fatty acid composition

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (9*cis*,12*cis* octadecadienoic acid), containing a conjugated double bond system. The predominant CLA isomer is rumenic acid (9*cis*, 11*trans* octadecadienoic acid) which represents 90 % of CLA present in milk and 75 % of CLA present in beef fat (Chin *et al.* 1992). Rumenic acid was identified as an intermediate in the biohydrogenation of linoleic acid by the rumen bacterium *Butyrivibrio fibrisolvens* (Viviani, 1970). Consequently, ruminant products show relatively high concentrations of CLA (0·5–1·5 % of total fatty acids), whereas meats from monogastric animals are poor sources of these compounds (0·1–0·2 % of total fatty acids; Chin *et al.* 1992). The ability of humans to synthetize CLA from linoleic acid directly is equivocal (Herbel *et al.* 1998). Instead, the bioconversion of *trans*-vaccenic acid to CLA in man was suggested (Santora *et al.* 2000).

Interest in CLA has increased in the past decade as a result of its potential beneficial health effects. Indeed, CLA was found to act as a growth factor (Chin *et al.* 1994), and a fat-to-lean repartitioning agent (Pariza *et al.* 1996; Park *et al.* 1997; Ostrowska *et al.* 1999), and to show anticarcinogenic (Schulz *et al.* 1992; Ip, 1997), hypocholesterolaemic and antiatherogenic (Lee *et al.* 1994; Nicolosi *et al.* 1997) properties. However, its role in the development of atherosclerosis is equivocal and needs further studies (Munday *et al.* 1999). CLA was also involved in stimulating the immune functions in chickens and rats (Cook *et al.* 1993). These immunostimulatory effects of CLA have been recently confirmed (Wong *et al.* 1998;

Abbreviations: CLA, conjugated linoleic acid; HDL-C, HDL-cholesterol; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol.

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Hayek *et al.* 1999) in experiments using young and old mice. In humans, milk fat consumption as the major source of CLA (Jiang *et al.* 1999), was demonstrated to protect against the risk of breast cancer in women (Knekt *et al.* 1996). Also, CLA can inhibit the growth of human breast cancer cells (Visonneau *et al.* 1997) and prostate cancer cells (Cesano *et al.* 1998), when implanted into immune-deficient mice.

In view of the above health-related effects of CLA it seems desirable to provide CLA-enriched products for human consumption. It has been already demonstrated that CLA is readily incorporated in tissue lipids in mice (Belury & Kempa-Steczko, 1997), rats (Chin *et al.* 1994; Sugano *et al.* 1997; Szymczyk *et al.* 2000), hamsters (de Deckere *et al.* 1999), and pigs (Kramer *et al.* 1998). The objective of the present study was to compare growth performance, feed conversion efficiency and carcass composition (fatty acid profiles) in broiler chickens fed commercial diets supplemented with either sunflower oil or graded levels of CLA. In addition, limited studies were conducted on serum lipoproteins in the chickens, in relation to intakes of CLA.

Material and methods

A total of 160 1 d old (eighty male and eighty female) chickens of a commercial strain (Arbor Acres), obtained from a local hatchery, were housed in electrically heated battery brooders, and 24 h of light were provided. The birds were given access *ad libitum* to water and to the control starter diet, containing 4 % of sunflower oil, for 7 d (Table 1). On day 8, the chickens were randomly allocated to group cages. They were given a starter diet from 7 to 21 d and a grower-finisher diet from 22 to 42 d (Table 1). The four dietary treatments consisted of four graded levels (0·0, 0·83, 1·66, 2·5 %) of the commercial CLA product (Table 2) containing 60 % CLA isomers (Natural Lipids Ltd., Hovdebygda, Norway). Thus, the resulting CLA dietary concentrations were 0·0, 0·5, 1·0, 1·5 % respectively. There were five replicates for each of four treatments, and each replicate cage contained eight broiler chickens. Body weights were recorded for each replicate on days 8, 22, and 42, and feed intake was measured over these periods in order to calculate feed conversion efficiency.

Table 1. Composition and nutrient content of standard experimental diets (g/kg)

Item	Diets	
	Starter	Grower-finisher
Ground maize	477	500
Ground wheat	90	125
Soybean meal	350	290
Sunflower oil*	40	40
Limestone	10	11
Dicalcium phosphate	20	21
NaCl	3	3
Mineral and vitamin premix†	10	10
In 1 kg of diet‡		
Crude protein (g)	215·0	193·6
Met (g)	4·7	4·0
Lys (g)	11·0	9·6
ME (MJ)	12·6	12·8
Crude fibre (g)	27·3	26·7
Ca (g)	9·3	9·6
P avail. (g)	4·2	4·2

* The CLA product (60 %) was substituted for sunflower oil (0·83, 1·66, 2·5 %) to obtain three experimental (0·5, 1·0, 1·5 % CLA) and control (0·0 CLA) groups.

† Premix provided per kg of diet: DKA-Starter: Vit. A, 8000 IU; Vit. D_3, 1200 IU; Vit. E, 10 mg; Vit. K_3, 2 mg; Vit. B_1, 1·5 mg; Vit. B_2, 4 mg; Vit. B_6, 0·3 mg; Vit. B_{12} 10 μg; D-calcium panthotenate, 8 mg; folic acid, 0·2 mg; nicotinamide, 12 mg; choline chloride, 150 mg; Mn, 50 mg; J, 0·3 mg; Zn 30 mg; Co, 0·4 mg; Se, 0·1 mg,; DL-methionine, 1 g. DKA-Grower/Finisher: Vit. A, 7000 IU; Vit. D_3, 1000 IU; Vit. E, 10 mg; Vit. K_3, 1·5 mg; Vit. B_2, 4 mg; Vit. B_6, 0·3 mg; Vit. B_{12} 10 μg, 10; D-calcium panthotenate, 3 mg; folic acid, 0·2 mg; nicotinamide, 10 mg; choline chloride, 200 mg; Mn, 50 mg; J, 0·2 mg; Zn, 30 mg; Co, 0·3 mg; Se, 0·2 mg; DL-methionine, 1 g.

‡ Calculated nutrient content.

Table 2. Fatty acid composition of the sunflower oil and the CLA supplement used in experimental diets (relative %)

Fatty acid	Sunflower oil	CLA
12:0	–	0·1
14:0	0·01	0·1
16:0	6·2	4·9
16:1	–	0·1
18:0	–	2·0
9*cis*-18:1	24·4	29·9
11*cis*-18:1	–	0·7
18:2	62·9	–
trans-18:2	–	0·8
9*cis*,11*cis*-18:2	–	0·5
CLA isomers (total):	–	58·7
9*cis*,11*trans*-18:2	–	9·5
8*trans*,10*cis*-18:2	–	8·6
11*cis*,13*trans*-18:2	–	9·8
10*trans*,12*cis*-18:2	–	11·2
Other CLA	–	19·6
18:3	1·0	–
20:0	0·3	0·9
22:0	0·6	0·3
24:0	0·2	0·1
Total fatty acids	95·6	99·1

At 42 d of age, eight birds (four male and four female) per each treatment, were stunned and slaughtered by neck cutting and exsanguinated. Blood samples were collected and serum samples were separated by low-speed centrifugation (1500 ***g*** for 15 min) to determine lipoprotein profiles. Serum total cholesterol (TC) was analysed enzymatically with standard kits (Sigma-Aldrich, Poznan Poland), according to Allain *et al.* (1974) and its HDL fraction (HDL-C) according to Warrick *et al.* (1982). The LDL fraction of cholesterol was calculated as a difference between TC and HDL-C. Triacylglycerol content was estimated according to McGowan *et al.* (1983).

Carcasses were then plucked, and eviscerated to determine carcass weight, as a percentage of total weight, and abdominal fat (considered to be the fat extending within the ischium, surrounding the cloaca, and adjacent to the abdominal muscle) and breast and leg muscle weight as a percentage of carcass weight. Samples of abdominal fat, breast (pectoralis major and pectoralis minor) and leg (flexor cruris medialis) muscles were stored frozen (−20°C) for further analysis.

The total tissue lipids (abdominal fat, breast and leg muscles) were extracted according to the method of Folch *et al.* (1957). They were saponified (10 min, 75°C) in 0·5 M KOH–methanol and then methylated (10 min, 75°C) in 14 % BF_3–methanol (Morrison & Smith, 1964). Finally,

Table 3. Effect of dietary CLA level on growth performance and slaughter characteristics of chickens
(Data are means for five replicates of each of four treatments for eight chickens per replicate)

Dietary level of CLA (%)†	Body weight gain (g) in periods (days)			Feed conversion efficiency‡ for periods (days)			Feed intake (kg) for periods (days)					Proportion (%) to mass of eviscerated carcass	
	8–21	22–42	8–42	8–21	22–42	8–42	8–21	22–42	8–42	Dressing percentage	Abdominal fat deposition (%)	Breast muscles	Leg muscles
0·0	468	1147	1615^{a}	0·600	0·455	0·488	$0·78^{A}$	2·52	$3·31^{a}$	72·9	$2·68^{a}$	21·7	$19·0^{a}$
0·5	456	1112	1568^{a}	0·585	0·434	0·469	$0·78^{A}$	2·56	$3·34^{a}$	73·1	$2·06^{ab}$	20·9	$20·6^{ab}$
1·0	461	1073	1534^{ab}	0·599	0·424	0·465	$0·77^{A}$	2·53	$3·30^{a}$	72·7	$1·78^{b}$	21·0	$21·3^{b}$
1·5	434	1002	1435^{b}	0·629	0·412	0·460	$0·69^{B}$	2·43	$3·12^{b}$	71·6	$1·94^{ab}$	21·1	$20·4^{ab}$
SEM	6·08	19·81	20·86	0·008	0·006	0·005	0·010	0·024	0·032	0·262	0·131	0·245	0·329
Contrast§													
Linear	NS	*	***	NS	*	*	***	NS	*	NS	*	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS

† Mean values for CLA dietary level effect with different superscripts are significantly different at $^{(a,b)}P < 0·05$ or $^{(A,B)}P < 0·01$.
‡ Feed conversion efficiency is body weight gain/feed intake.
§ Dietary CLA level effects were tested using linear and quadratic orthogonal contrasts. For details of procedures see p. 467. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$; NS $P \geq 0·05$.

fatty acid methyl esters were extracted with hexane and analysed on a Hewlett-Packard (model 5890) gas chromatograph, equipped with a BPX 70 fused silica capillary column (length 50 m × 0·22 mm i.d. × 0·25 mm film thickness; SGE International, Ringwood, Victoria, Australia), and a flame ionisation detector. Helium was the carrier gas used at a split ratio of 50:1. The operating conditions were as follows: the temperature of injector was 210°C, and that of detector was 240°C. The initial oven temperature was 160°C for 35 min, increasing progressively by 3°C/min to 210°C, and held constant at 210°C for 10 min. The fatty acid percentage was integrated and calculated using the HP ChemStation computer program. Fatty acid methyl esters were identified by comparison of their retention times with authentic standards purchased from Sigma-Aldrich (Poland) and the CLA reference standards (9*cis*,11*trans* and 10*trans*,12*cis* isomers) were obtained from Larodan Fine Chemicals AB (Malmõ, Sweden). All analyses were performed at the Meat and Fat Research Institute (Warsaw, Poland).

The isomeric distribution of CLA in tissue lipids was expressed as a percentage of total CLA isomers present in the tissue total fatty acids:

$$\text{CLA isomer profile (\%)} = \frac{\text{individual CLA isomer (\% of total FA)}}{\sum \text{CLA isomers (\% of total FA)}} \times 100.$$

The data were analysed using either one-way (growth performance and slaughter characteristics) or two-way (tissue fatty acid composition and serum lipoproteins) ANOVA generated by the STATISTICA *v.* 5·1 package. In addition, dietary CLA level effects were tested using linear and quadratic orthogonal contrasts. Where appropriate, Duncan's multiple range test was used to determine the significance of differences between treatment means at the $P < 0·05$ and $P < 0·01$ levels of significance. The relationship between dietary CLA content and total CLA concentration in tissue lipids was also analysed by a linear regression analysis.

Results

Food intake of broiler chickens (Table 3), was significantly ($P < 0·01$) decreased (from 0·78 to 0·69 kg) by dietary CLA over the starter (8–21 d) period, while no effects of CLA on this variable were noted over the grower-finisher (22–42 d) period. In spite of that, when determined for the total period 8–42 d, feed intake depression (from 3·31 to 3·12 kg) was found to be statistically significant ($P < 0·05$). This response was also linear ($P < 0·05$). Although body weight gains of birds (Table 3) were not affected significantly by dietary CLA over the starter (8–21 d) and the grower-finisher (22–42 d) period, they tended to decrease with increasing levels of CLA supplement. At the same time, when determined for the total period 8–42 d, body weight gains of broilers were significantly ($P < 0·05$) and linearly ($P < 0·01$) reduced (from 1615 to 1435 g). There were no significant effects of dietary CLA on feed conversion efficiency (Table 3), particularly in younger birds (8–21 d). However, older birds (22–42 d) decreased (from 0·455 to 0·412) this measurement linearly ($P < 0·05$). The same was true for the total experimental period 8–42 d, during which, feed conversion efficiency decreased from 0·488 to 0·460.

The carcass yield values, calculated on the basis of the carcass weight (after head and feet had been removed), and determined at the age of 42 d, showed no significant effects of dietary CLA on the dressing percentage (Table 3). Abdominal fat deposition was significantly and linearly ($P < 0·05$) reduced (from 2·68 for control to 1·94 % for the group fed 2 % dietary CLA respectively) whereas the relative proportion of breast and leg muscles (% of carcass weight) responded differently to increasing levels of dietary CLA. The former variable was not affected by the treatment and the latter was significantly ($P < 0·05$) increased.

Fatty acid composition of tissue lipids (abdominal fat, breast and leg muscles), expressed as a percentage of total

Table 4. Effect of dietary CLA (%) on relative (%) fatty acid composition of abdominal fat in experimental chickens: cockerels (♂) and pullets (♀) (Data are means for five replicates of each of four treatments for eight chickens per replicate)

	CLA dietary level (%)†				Contrast‡		Sex (S)			Significance of effects		
	0·0	0·5	1·0	1·5	L	Q	♂	♀	SEM (pooled)	%	S	%×S
14:0	$0·37^{A}$	$0·54^{B}$	$0·71^{C}$	$0·77^{C}$	***	NS	0·58	0·62	0·030	**	NS	NS
16:0	$17·84^{A}$	$22·17^{B}$	$26·00^{C}$	$26·15^{C}$	NS	***	22·78	23·28	0·667	**	NS	NS
7*cis*-16:1	$0·34^{A}$	$0·21^{B}$	$0·27^{AB}$	$0·30^{A}$	***	***	0·27	0·29	0·011	**	NS	NS
9*cis*-16:1	$2·47^{A}$	$1·31^{B}$	$1·30^{B}$	$1·00^{B}$	***	**	1·50	1·54	0·115	**	NS	NS
18:0	$6·10^{A}$	$10·74^{B}$	$13·10^{C}$	$13·96^{C}$	***	***	10·72	11·22	0·566	**	NS	NS
9*cis*-18:1	$33·09^{A}$	$27·20^{B}$	$26·00^{B}$	$27·10^{B}$	***	***	28·54	28·15	0·537	**	NS	NS
11*cis*-18:1	$1·36^{A}$	$0·90^{B}$	$0·85^{B}$	$1·05^{C}$	***	***	1·03	1·05	0·039	**	NS	NS
18:2	$36·16^{A}$	$32·21^{B}$	$23·51^{C}$	$17·72^{D}$	***	NS	27·55	27·29	1·350	**	NS	NS
18:3*n*-3	$0·76^{A}$	$0·66^{B}$	$0·60^{BC}$	$0·55^{C}$	***	NS	0·64	0·65	0·018	**	NS	NS
18:3*n*-6	$0·20^{A}$	$0·10^{B}$	$0·07^{B}$	$0·01^{C}$	***	NS	0·09	0·10	0·013	**	NS	NS
CLA isomers	$0·00^{A}$	$2·94^{B}$	$6·66^{C}$	$10·20^{D}$	***	NS	5·17	4·73	0·700	**	NS	NS
20:0	$0·11^{A}$	$0·17^{A}$	$0·16^{A}$	$0·27^{B}$	***	NS	0·18	0·18	0·013	**	NS	NS
20:1	$0·24^{A}$	$0·27^{AB}$	$0·29^{AB}$	$0·34^{B}$	***	NS	0·27	0·29	0·010	**	NS	NS
20:4	$0·19^{A}$	$0·10^{B}$	$0·06^{BC}$	$0·01^{C}$	***	NS	0·09	0·09	0·014	**	NS	NS
Total SFA	$24·62^{A}$	$33·82^{B}$	$40·40^{C}$	$41·36^{C}$	***	***	34·56	35·54	1·238	**	NS	**
Total MUFA	$37·60^{A}$	$29·90^{B}$	$28·71^{B}$	$29·57^{B}$	***	***	31·65	31·35	0·679	**	NS	**
Total PUFA	$37·41^{A}$	$36·24^{A}$	$31·11^{B}$	$28·57^{B}$	***	NS	33·69	33·07	0·801	**	NS	**

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; L, linear; Q, quadratic; S, sex.
† Mean values for CLA dietary level effect with different superscripts are significantly different at $P < 0·01$.
‡ For details of statistical tests see p. 467. ** $P < 0·01$; *** $P < 0·001$; NS $P \geq 0·01$.

methyl esters of fatty acids, was significantly altered by increasing dietary CLA concentrations (Tables 4, 5 and 6); the effect of sex was not detected. Generally, no CLA was found in the tissue lipids of broilers fed the control diet. In contrast, the concentration of CLA isomers was increased linearly ($P < 0·001$) in tissue samples (abdominal fat: 2·94, 6·66, and 10·20 %; breast: 2·89, 5·25, and 9·35 %; leg: 3·22, 5·46, and 10·27 %) from broilers fed 0·5, 1·0, and 1·5 % dietary CLA. No significant quadratic responses (except leg muscles) were detected. The relationship between dietary and tissue CLA concentrations are given in Fig. 1. Fatty acid profiles of tissue lipids did not reflect that of the commercial CLA product, thus indicating preferential incorporation of CLA isomers. Generally, the relative proportions of 9*cis*,11*trans* and 11*cis*,13*trans* CLA isomers in tissue lipids exceeded those found in the CLA

Table 5. Effect of dietary CLA (%) on relative (%) fatty acid composition of breast muscles in experimental chickens: cockerels (♂) and pullets (♀) (Data are means of five replicates per treatment for eight chickens per replicate)

	CLA dietary level (%)†				Contrast‡		Sex (S)			Significance of effects		
	0·0	0·5	1·0	1·5	L	Q	♂	♀	SEM (pooled)	%	S	%×S
14:0	$0·31^{A}$	$0·42^{AB}$	$0·51^{B}$	$0·55^{B}$	***	NS	0·43	0·47	0·022	**	NS	NS
16:0	$19·47^{A}$	$22·21^{B}$	$24·56^{C}$	$24·59^{C}$	***	**	22·21	23·21	0·436	**	NS	NS
7*cis*-16:1	0·30	0·22	0·24	0·22	**	NS	0·25	0·24	0·010	NS	NS	NS
9*cis*-16:1	$1·76^{A}$	$1·01^{B}$	$0·97^{B}$	$0·75^{B}$	***	NS	1·14	1·11	0·096	**	NS	NS
17:0	0·14	0·10	0·10	0·10	**	NS	0·11	0·11	0·005	NS	NS	NS
18:0	$8·87^{A}$	$11·77^{B}$	$13·12^{BC}$	$13·71^{C}$	***	***	11·71	12·03	0·396	**	NS	NS
9*cis*-18:1	25·00	22·09	20·05	21·10	NS	NS	21·92	22·20	0·521	NS	NS	NS
11*cis*-18:1	$1·95^{A}$	$1·12^{B}$	$1·00^{B}$	$0·95^{B}$	***	NS	1·26	1·25	0·077	**	NS	NS
18:2	$30·70^{A}$	$30·11^{A}$	$26·79^{AB}$	$23·17^{B}$	***	NS	28·43	26·95	0·729	**	NS	NS
18:3 *n*-3	0·52	0·46	0·40	0·46	NS	NS	0·46	0·47	0·026	NS	NS	NS
18:3 *n*-6	$0·19^{A}$	$0·14^{A}$	$0·04^{B}$	$0·01^{B}$	***	NS	0·09	0·09	0·015	**	NS	NS
CLA isomers	$0·00^{A}$	$2·89^{B}$	$5·25^{C}$	$9·35^{D}$	***	NS	4·46	4·29	0·647	**	NS	NS
20:0	$0·07^{A}$	$0·10^{AB}$	$0·11^{AB}$	$0·15^{B}$	***	NS	0·11	0·11	0·008	**	NS	NS
20:1	$0·26^{A}$	$0·26^{A}$	$0·30^{AB}$	$0·39^{B}$	***	NS	0·31	0·29	0·014	**	NS	NS
20:2	$0·89^{A}$	$0·47^{B}$	$0·40^{B}$	$0·25^{B}$	***	NS	0·51	0·50	0·058	**	NS	NS
20:3	0·75	0·56	0·76	0·59	NS	NS	0·69	0·64	0·052	NS	NS	NS
20:4	$5·55^{A}$	$3·81^{AB}$	$3·25^{AB}$	$1·84^{B}$	***	NS	3·61	3·62	0·416	**	NS	NS
20:5	$0·00^{A}$	$0·00^{A}$	$0·22^{B}$	$0·30^{B}$	***	*	0·14	0·12	0·025	**	NS	NS
22:4	$1·65^{A}$	$1·00^{AB}$	$0·66^{B}$	$0·27^{B}$	***	NS	0·86	0·93	0·128	**	NS	NS
22:5	0·35	0·27	0·34	0·20	NS	NS	0·31	0·27	0·028	NS	NS	NS
22:6	0·46	0·30	0·24	0·21	**	NS	0·30	0·31	0·034	NS	NS	NS
Total SFA	$28·97^{A}$	$34·71^{B}$	$38·51^{C}$	$39·20^{C}$	***	**	34·67	36·02	0·814	**	NS	**
Total MUFA	29·34	34·72	22·56	23·41	**	NS	29·92	25·10	2·555	NS	NS	*
Total PUFA	$41·06^{a}$	$40·02^{ab}$	$38·35^{ab}$	$36·66^{b}$	**	NS	39·84	38·20	0·645	*	NS	*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; L, linear; Q, quadratic; S, sex.
† Mean values for CLA dietary level effect with different superscripts are significantly different at $^{(A,B,C)}P < 0·01$ or at $^{(a,b)}P < 0·05$.
‡ For details of statistical tests see p. 467. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$; NS $P \geq 0·05$.

Table 6. Effect of dietary CLA (%) on relative (%) fatty acid composition of leg muscles in experimental chickens: cockerels (♂) and pullets (♀)
(Data are means of five replicates per treatment for eight chickens per replicate)

	CLA dietary level (%)†				Contrast‡		Sex (S)			Significance of effects		
	0·0	0·5	1·0	1·5	L	Q	♂	♀	SEM (pooled)	%	S	%×S
14:0	$0·36^{A}$	$0·49^{B}$	$0·54^{BC}$	$0·64^{C}$	***	NS	0·48	0·53	0·022	**	NS	NS
16:0	$18·35^{A}$	$21·30^{B}$	$23·05^{BC}$	$23·92^{C}$	***	NS	21·17	22·13	0·461	**	NS	NS
7*cis*-16:1	$0·35^{A}$	$0·26^{B}$	$0·25^{B}$	$0·27^{AB}$	**	**	0·29	0·28	0·011	**	NS	NS
9*cis*-16:1	$2·45^{A}$	$1·27^{B}$	$1·06^{B}$	$0·84^{B}$	NS	**	1·48	1·33	0·135	**	NS	NS
17:0	0·14	0·17	0·15	0·14	***	NS	0·13	0·17	0·009	NS	NS	NS
18:0	$8·65^{A}$	$11·45^{B}$	$14·46^{C}$	$13·89^{C}$	***	**	11·77	12·45	0·471	**	NS	NS
9*cis*-18:1	$26·59^{A}$	$22·92^{B}$	$20·04^{C}$	$21·87^{BC}$	***	**	23·37	22·34	0·567	**	NS	**
11*cis*-18:1	$1·61^{A}$	$0·99^{B}$	$0·91^{B}$	$0·95^{B}$	***	***	1·10	1·13	0·056	**	NS	NS
18:2	$33·36^{A}$	$31·34^{A}$	$27·06^{B}$	$22·26^{C}$	***	NS	28·99	28·02	0·872	**	NS	NS
18:3 *n*-3	$0·70^{A}$	$0·69^{A}$	$0·52^{B}$	$0·55^{AB}$	***	NS	0·62	0·61	0·022	**	NS	NS
18:3 *n*-6	$0·21^{A}$	$0·19^{A}$	$0·05^{B}$	$0·01^{B}$	***	NS	0·11	0·12	0·017	**	NS	NS
CLA isomers	$0·00^{A}$	$3·22^{B}$	$5·46^{C}$	$10·27^{D}$	***	**	4·82	4·66	0·686	**	NS	NS
20:0	0·11	0·12	0·11	0·12	NS	NS	0·12	0·12	0·007	NS	NS	NS
20:1	$0·25^{A}$	$0·27^{A}$	$0·30^{A}$	$0·37^{B}$	***	NS	0·31	0·29	0·012	**	NS	NS
20:2	$0·47^{A}$	$0·34^{B}$	$0·30^{B}$	$0·16^{C}$	***	NS	0·30	0·34	0·025	**	NS	NS
20:3	0·39	0·25	0·46	0·41	NS	NS	0·37	0·39	0·025	NS	NS	NS
20:4	$3·87^{A}$	$2·60^{AB}$	$3·26^{AB}$	$1·80^{B}$	*	NS	2·64	3·13	0·261	**	NS	NS
20:5	$0·00^{A}$	$0·00^{A}$	$0·07^{B}$	$0·21^{C}$	***	***	0·08	0·06	0·017	**	NS	NS
22:4	$0·92^{A}$	$0·54^{B}$	$0·54^{B}$	$0·24^{B}$	***	NS	0·50	0·62	0·062	**	NS	NS
22:5	0·22	0·16	0·25	0·20	NS	NS	0·19	0·22	0·016	NS	NS	NS
22:6	0·26	0·20	0·20	0·16	NS	NS	0·18	0·23	0·019	NS	NS	NS
Total SFA	$27·71^{A}$	$33·63^{B}$	$38·41^{C}$	$38·81^{C}$	***	***	33·78	35·50	0·873	**	*	**
Total MUFA	$31·60^{A}$	$25·86^{B}$	$22·67^{C}$	$24·41^{BC}$	***	***	26·74	25·53	0·742	**	NS	**
Total PUFA	$40·42^{a}$	$39·52^{a}$	$38·18^{ab}$	$36·29^{b}$	**	NS	38·81	38·40	0·565	*	NS	*

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; L, linear; Q; quadratic; S, sex.
† Mean values for CLA dietary level effect with different superscripts are significantly different at $^{(A,B,C)}P < 0·01$ or at $^{(a,b)}P < 0·05$.
‡ See text for more details of statistical tests. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$; NS $P \geq 0·05$.

product. On the other hand, 8*trans*, 10*cis* and 10*trans*, 12*cis*, and other CLA isomers were incorporated into tissue lipids less efficiently (Table 7). With regard to total fatty acid profiles, the relative proportions of saturated fatty acids were significantly ($P < 0·01$) increased, due mainly to the increase in the concentration of palmitic (16:0) and stearic (18:0) acid. Saturated fatty acids responded also linearly ($P < 0·001$) and quadratically ($P < 0·01$). At the same time, the relative proportions of monounsaturated fatty acids (MUFA) in abdominal fat and leg muscles were significantly ($P < 0·01$) reduced. MUFA in these tissues responded also in a linear ($P < 0·01$) and quadratic ($P < 0·001$) manner. Polyunsaturated fatty acids (PUFA) were significantly ($P < 0·05$) and linearly ($P < 0·01$) reduced. These latter changes resulted mainly from the fall in the concentration of palmitoleic (16:1), oleic (18:1), linoleic (18:2) and arachidonic (20:4) acid (see: arachidonate in breast and leg muscles; Tables 5 and 6). Although present in negligible concentrations, the relative proportions of linolenic (18:3) acid were decreased (Tables 4, 5 and 6). In addition, concentrations of eicosapentaenoic (20:5) acid were increased and those of docosahexaenoic (22:6) decreased in breast and leg muscles (Tables 5 and 6) with increasing levels of dietary CLA. Generally, these responses were linear.

Serum lipoproteins were significantly affected by both treatments (dietary CLA and sex of birds; Table 8). TC concentrations reached a maximum (141·73 mg/dl) in broilers fed 1·0 % CLA and then slightly decreased ($P < 0·01$), resulting in a linear ($P < 0·01$) and quadratic ($P < 0·05$) effect. The same was true also for HDL-C responding in a quadratic ($P < 0·05$) manner. The resulting HDL-C:TC

Table 7. Comparison of CLA isomer profile (%) of commercial CLA preparation, abdominal and muscle fat of experimental chickens

		Abdominal fat				Breast muscle				Leg muscle			
		Dietary level of CLA (%)				Dietary level of CLA (%)				Dietary level of CLA (%)			
	CLA preparation	0·0	0·5	1·0	1·5	0·0	0·5	1·0	1·5	0·0	0·5	1·0	1·5
CLA isomer:													
9*cis*,11*trans*-18:2	16·18	–	19·04	20·50	20·00	–	17·64	18·85	19·89	–	18·94	19·23	20·35
8*trans*,10*cis*-18:2	14·66	–	11·90	9·90	10·58	–	8·65	6·86	8·56	–	10·87	7·51	8·47
11*cis*,13*trans*-18:2	16·69	–	23·80	23·12	22·65	–	29·76	35·52	28·56	–	27·02	30·04	26·97
10*trans*,12*cis*-18:2	19·08	–	19·04	18·62	18·72	–	15·22	15·24	16·26	–	14·90	15·93	16·85
Other CLA	33·39	–	25·85	27·77	27·94	–	25·95	25·52	25·24	–	29·19	27·29	27·26

$$\text{CLA isomer profile } (\%) = \frac{\text{individual CLA isomer } (\%\text{ of total FA})}{\sum \text{ CLA isomers } (\%\text{ of total FA})} \times 100.$$

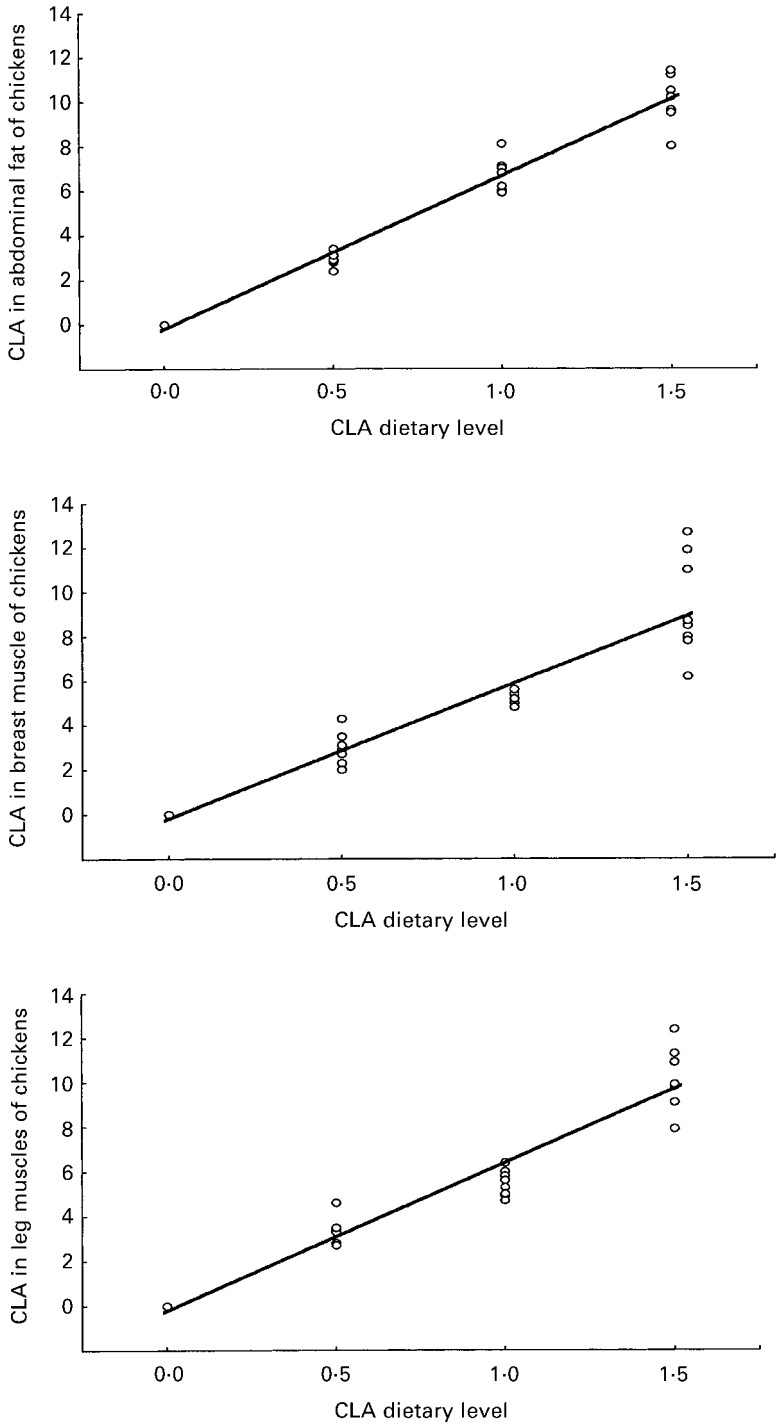

Fig. 1. Relationship between dietary CLA isomers content (g/100 g diet) and proportions of CLA isomers (g/100 g total fatty acids) in abdominal fat (AF), breast (BM) and leg muscles (LM) of chickens. CLA dietary level *v.* CLA in AF, r 0·98 and $P = 0{\cdot}000000$; *v.* CLA in BM, r 0·94 and $P = 0{\cdot}000000$; *v.* CLA in LM, r 0·97 and $P = 0{\cdot}000000$.

tended to decrease (from 0·82 to 0·79). Although serum triacylglycerol concentration tended to increase (from 36·75 to 40·14 mg/dl), there was no significant effect of dietary CLA supplementation on this variable. Moreover, serum concentrations of TC and HDL-C were significantly ($P < 0{\cdot}01$) higher in males than in females.

Discussion

Depressed growth performance in broilers, resulting from feeding diets containing 0·0, 0·5, 1·0, and particularly 1·5 % dietary CLA, was consistent with earlier findings on mice (Belury & Kempa-Steczko, 1997) and rats (Szymczyk

Table 8. Effect of dietary CLA (%) on serum lipoprotein in experimental chickens: cockerels (♂) and pullets (♀)

	Dietary level of CLA (%)†				Contrast‡		Sex (S)			Significance of effects		
	0·0	0·5	1·0	1·5	L	Q	♂	♀	SEM (pooled)	%	S	%×S
Total cholesterol (mg/dl)	$117{\cdot}39^{A}$	$137{\cdot}41^{B}$	$141{\cdot}73^{B}$	$136{\cdot}47^{B}$	**	*	144·23	122·27	1·12	**	**	*
HDL-cholesterol (mg/dl)	$96{\cdot}85^{A}$	$112{\cdot}54^{B}$	$113{\cdot}58^{B}$	$109{\cdot}97^{B}$	NS	*	117·16	99·31	1·42	**	**	*
HDL-cholesterol:	0·82	0·82	0·79	0·80	NS	NS	0·81	0·81	0·007	NS	NS	NS
Triacylglycerols (mg/dl)	36·75	34·73	35·35	40·14	NS	NS	38·89	34·59	1·12	NS	NS	NS

L, linear; Q, quadratic; S, sex. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; NS $P \geq 0{\cdot}05$.
† Mean values in the same row with different superscripts are significantly different at $^{(A,B)}P < 0{\cdot}01$.
‡ For details of statistical tests see p. 467.

et al. 2000), fed the 1·5 % CLA-supplemented diet. In both studies marked reduction of growth variables (weight gains and feed conversion efficiency), relative to control animals, was observed. At the same time, our findings contrasted with those obtained earlier by Chin *et al.* (1994) in growing rats fed CLA. In the above work, the 0·25 and 0·50 % CLA-supplemented diets enhanced feed intake, weight gains and feed conversion efficiency (in males and females) relative to control animals. Similarly, pigs fed diets containing 0·0–0·5 % dietary CLA, tended to decrease feed intake and to increase weight gains, with concurrent significant improvements in feed conversion efficiency (Ostrowska *et al.* 1999). These data together suggest that dietary CLA has a potent effect on animal body mass resulting, most probably, from alterations in whole body metabolism. Thus, CLA was reported as a potent inhibitor of body fat accumulation in mice, rats, and chickens (Pariza *et al.* 1996). It was reported also to act as a fat-to-lean repartitioning agent in growing pigs (Dugan *et al.* 1997; Ostrowska *et al.* 1999). Consequently, these alterations in the ratio of fat-to-lean lead generally to improvements in feed conversion efficiency. However, at relatively high dietary CLA concentrations (exceeding 1·0 %), the beneficial changes in body composition do not bring expected improvements in growth performance. The possible explanation is that an excessive CLA treatment may stimulate fatty acid oxidation and thus enhance metabolic rate in animals, as demonstrated in mice (West *et al.* 1998).

The changes in broiler carcass composition were, at least partly, comparable with similar alterations reported earlier in pigs fed CLA-supplemented diets. Significantly reduced deposition of abdominal fat in broilers was in line with fat-to-lean repartition, reduced back fat thickness and lower fat contents of commercial meat cuts in pigs fed CLA (Dugan *et al.* 1997; Dunshea *et al.* 1998; Thiel *et al.* 1998). In the same line, although the relative proportion (% of carcass weight) of the breast muscles was not altered, that of the leg muscles was significantly ($P < 0{\cdot}05$) decreased, confirming further favourable effects of CLA on animal carcass composition. These effects may be attributed to the ability of CLA to reduce body fat accretion with concurrent increases in lean tissue deposition as reported already in mice (Park *et al.* 1997) and more recently in pigs (Ostrowska *et al.* 1999). To offer an explanation, CLA has been found to reduce lipoprotein lipase activity and to increase lipolysis in cultured murine adipocytes (Park *et al.* 1997), thus leading to reduced fat deposition and favourably altered body composition.

As could be expected, feeding incremental levels of dietary CLA (0·0–1·5 %) resulted in linear increases in concentrations of CLA isomers in tissue lipids. Indeed, dietary CLA isomers were efficiently transferred in mice (Belury & Kempa-Steczko, 1997), rats (Chin *et al.* 1994; Sugano *et al.* 1997; Szymczyk *et al.* 2000), hamsters (de Deckere *et al.* 1999), and pigs (Kramer *et al.* 1998), to various classes of body lipids. Moreover, incorporation of individual CLA isomers into body lipids differed as indicated by preferential incorporation of 9*cis*,11*trans* and 11*cis*,13*trans* CLA at the expense of 8*trans*,10*cis* and 10*trans*,12*cis*, and other CLA isomers in our studies. Similarly, both 9*cis*,11*trans* and 10*trans*,12*cis* CLA isomers were incorporated into adipose tissue in rats (Sugano *et al.* 1997) and hamsters (de Deckere *et al.* 1999), but the former much more efficiently. Alternatively, there were no differences between the distribution of CLA isomers in the commercial CLA source (Natural Lipids Ltd.) fed to pigs, and in back fat, omental fat, liver and heart triacylglycerols (Kramer *et al.* 1998). Other striking results of our studies were the changes in the relative proportions of different classes of fatty acids in the abdominal fat, breast and leg muscles. Generally, the saturated fatty acid content of the above tissues was significantly ($P < 0{\cdot}01$) increased and that of the MUFA and PUFA decreased ($P < 0{\cdot}05$). These changes in the fatty acid profiles, were due mainly to increases in concentrations of 16:0 and 18:0, and concurrent opposite changes in concentrations of 16:1, 18:1, 18:2, and 20:4 (20:4 in breast and leg muscles). They could have resulted, at least partly, from the inhibition of Δ9 desaturase activity in the liver, caused by CLA, and impaired conversion of 18:0 to 18:1 (Lee *et al.* 1995, 1998). On the contrary, CLA increased 18:1 content in mouse liver lipids (Belury & Kempa-Steczko, 1997) and hamster fat pads (de Deckere *et al.* 1999). The reduction in 18:2 and 20:4 concentrations is difficult to explain. However, it could be related to changes in 18:2, known to act as a metabolic precursor of 20:4. In fact, CLA gradually replaced sunflower oil (a rich source of linoleate) in the experimental diets. Correspondingly, dietary CLA decreased linoleate and arachidonate in mice (Belury & Kempa-Steczko, 1997) and hamsters (de Deckere *et al.* 1999) liver lipids, rat adipose and muscle tissue (Szymczyk *et al.* 2000), and hamster fat pads (de Deckere *et al.* 1999). The reduction in arachidonate concentration has received particular attention as arachidonate-derived eicosanoids (prostaglandin E_2, in particular) are frequently associated with the enhancement of cancer development (Belury & Kempa-Steczko, 1997; Liu & Belury, 1998). Finally, the changes in concentrations of

18:3 and derived 20:5 (eicosapentaenoic acid) and 20:6 (docosahexaenoic acid) were equivocal. With apparent reduction in linolenate, the eicosapentaenoic acid concentrations were increased and those of docosahexaenoic acid decreased. We have no explanation for these effects.

Significant increases in serum TC concentrations, contradicted earlier results of different authors indicating hypocholesterolaemic effect of dietary CLA in different animal models (Lee *et al.* 1994; Nicolosi *et al.* 1997; Munday *et al.* 1999; Szymczyk *et al.* 2000). At the same time, the elevated concentrations of HDL-C were generally consistent with the above findings. However, the resulting ratio HDL-C:TC tended to decrease, indicative of an unfavourable lipoprotein profile (Griffin, 1999). Although insignificant, the increased concentrations of atherogenic triacylglycerols in our studies, confirmed previous findings of Szymczyk *et al.* (2000) on rats. Also, the CLA treatment resulted in elevated concentrations of serum non-esterified fatty acids in growing pigs (Ostrowska *et al.* 1999). This effect may result from inhibitory action of CLA on lipoprotein lipase coupled with stimulation of lipolysis in adipose tissue (Park *et al.* 1997). Consequently, reduced fat deposition and increased lipolysis in adipocytes could be responsible for elevated serum triacylglycerol concentrations, as found in broiler chickens. Interestingly, significant decreases in serum triacylglycerol concentrations in mice fed the 0·25 % and 0·50 % CLA-supplemented diets were reported by Munday *et al.* (1999).

In conclusion, our studies show that feeding CLA in incremental dietary concentrations (0·0–1·5 %) to broilers is an effective way to obtain CLA-enriched poultry meat and thus the potential health-related benefits of CLA consumption in humans. At the same time, the broilers fed CLA-supplemented diets exhibit impaired growth performance. This negative effect is apparent particularly in broilers fed the 1·5 % CLA-supplemented diet. Of the other effects, the deposition of abdominal fat is favourably reduced, the relative proportion of breast muscles (% of carcass weight) is unaffected and that of leg muscles increased. The CLA supplement adversely affects the fatty acid composition of these tissues by increasing their saturated fatty acid content (e.g. 16:0, 18:0) at the expense of monounsaturated (e.g. 16:1, 18:1) and polyunsaturated (e.g. 18:2, 20:4) fatty acids. Further studies are certainly required to determine the optimum dietary concentration and balance of CLA isomers needed to obtain CLA-enriched poultry meat and to avoid potentially adverse effects of CLA supplementation. In the context of our results it can be theoretically calculated that in order to provide the minimum required amount of CLA (1·5 g/d; Decker, 1995), a 70 kg man would have to consume either a 1875 g portion of broiler breast or 300 g portion of broiler leg meat, obtained from birds fed the 1·5 % CLA-supplemented diet. The same amount of CLA would be provided by 8·5 kg of 3·5 % milk (Fritche & Steinhart, 1998).

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