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The effect of dietary conjugated linoleic acid on the levels of lipids, cholesterol and iodothyronines in the blood of pigs

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ABSTRACT

The aim of the investigation was to study the effect of dietary conjugated linoleic acid (CLA) on the concentrations of lipids, fatty acids and iodothyronines: thyroxine (T_4), triiodothyronine (T_3), reverse-triiodothyronine (rT_3), free T_4 (FT_4) and free T_3 (FT_3) in blood plasma of pigs. The experiment was carried out on 50 fatteners (average liveweight 50 kg) divided into 5 groups (5 gilts and 5 barrows per group). The pigs were fed a basal diet supplemented with 0, 0.5, 1, 1.5 and 2% of CLA isomers. Blood samples were collected from fatteners weighing 105 kg. Supplementation of the diet with CLA did not significantly affect lipid parameters in blood plasma. In comparison with gilts, a significantly higher concentration of triglycerides in barrows was found. In CLA-fed pigs, significant changes in the plasma fatty acid profile were noticed. The higher doses of CLA caused a significant increase in CLA and saturated fatty acids (SFA) with a concomitant decrease in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in blood plasma. Moreover, dietary CLA decreased the Δ9 desaturase index in a dose-dependent manner, probably as a result of

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the inhibitory effect of CLA on stearoyl-CoA activity.Only the lowest dose of CLA (0.5%) increased T_4 and T_3 concentrations in blood plasma of fatteners by 64 and 27%, respectively. There were no significant changes in rT_3, FT_4 and FT_3 in the plasma of CLA-treated pigs. The results obtained indicate that dietary CLA influenced the fatty acid profile in blood plasma of fatteners without affecting basic lipid parameters. The increase in T_4 and T_3 concentrations in the blood of CLA-treated pigs suggests that low doses of CLA may stimulate the function of the pituitary-thyroid axis and/or deiodinase activity. On the other hand, the absence of changes in FT_4 and FT_3 indicates that CLA treatment does not affect thyroid hormone homeostasis in blood.

KEY WORDS: fatteners, CLA, blood plasma, fatty acids, lipid indicators, thyroid hormones

INTRODUCTION

Conjugated linoleic acid (CLA) comprises an isomeric mixture of linoleic acids [18:2(n-6)] that are polyunsaturated fatty acids differing in the positions and configuration of the conjugated double-bond pairs. Recent years have seen a growing interest in the effect of CLA on animal metabolism and productivity (Dugan et al., 2004). Interest in these investigations has significantly increased in association with the results of *in vivo* and *in vitro* studies showing marked physiological activity of CLA that is of great importance in normal body function and health. It has been shown that CLA has antioxidant and antiobesity activity that leads to body weight reduction (DeLany and West, 2000). In other studies, CLA has been shown to protect against atherosclerosis and to exert a hypocholesterolaemic effect (Corino et al., 2002). In a wide range of animal models it has been demonstrated that CLA exerts anticarcinogenic activity (Belury, 2002). Dietary CLA supplementation has been revealed to increase the activity and function of the immune system (Sugano et al., 1997) and to attenuate the negative effects of inflammatory reactions by inhibiting mRNA expression and production of proinflammatory cytokins by blood cells (Changhua et al., 2004).

In pigs the potential effect of conjugated linoleic acid on the rate of fat deposition in adipose tissue has been well documented (DeLany and West, 2000; Corino et al., 2002; Ostrowska et al., 2003). The mechanism of this phenomenon is attributed to the direct effect of CLA on the activity of key enzymes involved in mobilization and deposition of fat in adipocytes (Park et al., 1997). The effect of CLA on adipose tissue metabolism can be better understood by determining the blood concentration of some lipid fractions, fatty acids and hormones in blood plasma and the activity of enzymes involved in lipid metabolism in the adipose tissue of animals fed a diet supplemented with CLA isomers. One of these enzymes is Δ9-desaturase stearoyl-CoA, which is responsible for fatty acid synthesis, mainly monounsaturated fatty acids (MUFA). Corl et al. (2001) have proposed estimation of a desaturase index as the ratio between MUFA and the sum

of saturated fatty acids (SFA) and MUFA. It has been shown that dietary CLA supplementation decreased the desaturase index as the result of the attenuation of the activity of Δ9-desaturase stearoyl-CoA (Smith et al., 2002).

Thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), belong to the most essential regulators of lipids metabolism in adipose tissue and their concentrations in blood plasma. These hormones affect the basal metabolic rate and thermogenic processes (Silva, 1995); thyroid hormones exert anabolic or catabolic properties at low or high concentrations, respectively (Eberhardt et al., 1980). With specific consideration of lipid metabolism, iodothyronines have an effect on synthesis, mobilization and degradation of lipids, although degradation is influenced more than synthesis (Pucci et al., 2000). The most important effects of iodothyronines on lipid metabolism include: 1. enhanced utilization of lipid substrates, 2. increased synthesis and mobilization of triglycerides stored in adipose tissue, 3. increased concentrations of non-esterified fatty acids (NEFA), 4. augmented lipoprotein-lipase activity (Pucci et al., 2000). It has been established that in pigs, the blood concentrations of lipids and cholesterol depend on the animal breed, sex, age and nutrition (Barowicz et al., 2000). Recently, it has been shown that in fatteners during growth there is a strong relationship between genotype and thyroid activity with lipid and cholesterol levels in blood plasma. A gradual decrease in total lipids in blood plasma was correlated with a decrease in T_3, the main metabolic hormone, in blood plasma (Migdał et al., 2003).

In pigs there have been no reported studies concerning simultaneous determination of lipids and thyroid hormone concentrations in blood plasma following dietary CLA supplementation. Therefore, the present experiment was designed to estimate the influence of supplementing diets with increasing CLA levels on lipid levels (i.e. triglycerides, total cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions), fatty acid profile, and iodothyronine concentrations in blood plasma of fatteners. The main aim of the investigation was to study the interaction between dietary CLA and thyroid hormone activity to explain changes in lipid parameters in blood plasma. Moreover, the results of the experiment may provide valuable observations for human medicine concerning the effect of dietary CLA on lipid metabolism and thyroid function.

MATERIAL AND METHODS

Animals and management

The experiment was carried out on 50 fatteners of the Polish Large White breed with an initial body weight of 50±2 kg, kept in individual pens equipped with automatic drinkers. During the experiment the pigs were fed *ad libitum* complete mixtures.

Experimental design and treatment

Fatteners were randomly divided into 5 groups (5 gilts and 5 barrows per group). Treatment with CLA given at 0, 0.5, 1, 1.5 and 2% DM of the ration. Because of a similar higher fatty acid profile, a 2% sunflower oil supplement was used in the group given no CLA to balance the energy value of the complete diets (Table 1). Animals received a finisher diet used at Slaughter Pig Testing

Table 1. Fatty acids composition of conjugated linoleic acids (CLA) and sunflower oil (% of acids sum) applied in the experiment

Fatty acids	Sunflower oil	CLA
C 12:0	0.024	0.169
C 14:0	0.071	0.123
C 16:0	5.99	4.14
C 16:1 *n-7*	0.089	0.095
C 18:0	3.03	1.12
C 18:1 *n-9*	27.176	21.605
C 18:2 *n-6*	61.86	3.02
C 18:3 *n-6*	-	0.013
C 18:3 *n-3*	0.80	0.153
C 18:2 *c9t11*	0.024	20.978
C 18:2 *t10c12*	0.008	22.29
C 18:2 *c9c11*	0.008	18.53
C 18:2 *t9t11*	0.245	6.688
C 20:0	0.19	0.915
C 20:4 n-*6*	-	-
C 20:5 *n-3*	0.004	0.009
C 22:0	0.417	0.128
C 22:1	0.064	0.019
C 22:6 *n-3*	-	-
Sum of CLA	0.284	68.486

Stations that contains wheat, triticale, maize, soyabean meal, minerals, Lutamix premix (BASF, Kutno) and a CLA supplement (Edenor UKD 6010, Henkel). CLA contained the isomers C 18:2 *c*9*t*11 (20.9%), C 18:2 *t*10*c*12 (22.3%), C 18:2 *c*9*c*11 (18.5%) and C 18:2 *t*9*t*11 (6.7%). The control group received sunflower oil (ZPT, Warsaw) containing, %: SFA 6.6, MUFA 21.7 and PUFA 71.7 (0.16% of n-3 PUFA and 3.0% of n-6 PUFA). The metabolizable energy (13.2 MJ) was calculated based on the diet composition, assuming tabular values for individual components (Nutrient Requirements of Pigs, 1993). The basal and amino acid compositions of the diets were determined according to AOAC (1990). The composition of feed mixtures was, %: crude protein 16.2, crude fibre 3.3, crude fat 1.9, total phosphorus 0.63, calcium 0.91, lysine 0.9, methionine with cystine 0.57, and tryptophan 0.21 (Table 2). Throughout the

Table 2. Composition and nutritive value of feed mixtures

Item	Composition, %
Ingredient	
ground wheat	40.0
ground triticale	22.8
ground maize	20.0
soyabean meal (46% CP)	14.0
limestone	1.7
dicalcium phosphate	0.5
vitamin-mineral premix (Lutamix complete, BASF, Kutno, Poland)	1.0
Calculated nutritive value	
metabolizable energy, MJ	13.16
crude protein, %	16.15
crude fibre, %	3.30
crude fat, %	1.88
crude ash, %	4.72
Ca, %	0.91
total P, g	0.63
available P, %	0.36
Na, %	0.16
lysine, %	0.90
methionine+cystine, %	0.57
treonine, %	0.56
tryptophan, %	0.21

experiment, the animals were fed in accordance with the Nutrient Requirements of Pigs (1993). Blood was sampled at approximately 105 kg body weight from the anterior caval vein into heparinized tubes. Blood plasma was obtained after centrifuging whole blood at 3000 rpm for 10 min. Plasma was then transferred into vials and frozen at -28ºC.

Analytical procedures

The levels of triglycerides (TG), total cholesterol (TC) and HDL cholesterol were determined enzymatically using Cormay kits (Lublin, Poland). LDL cholesterol was calculated using the Friedewald formula: LDL = TC - HDL - TG/5 (Friedewald et al., 1972). The composition of plasma fatty acids was determined using gas chromatography according to procedure 975.39 (AOAC, 1990) after extraction with a methanol and chloroform mixture (v/v 1:2). Determinations were carried on a TRACE GC ULTRA gas chromatograph (Electron Co., USA) with a SUPELCOWAX 10 (30 m × 0.25 mm I.D. × 0.25 µm film thickeness) column under the following conditions: helium as the carrier gas (flow rate

5 ml/min), split flow 10 ml/min, injector temperature 220°C, column temperature 200°C, detector temperature 250°C.

The plasma level of thyroid hormones was determined by radioimmunoassay. Total T_4, free T_4 (FT_4) and free T_3 (FT_3) were determined using commercial CIS kits (US Inc., USA): RIA-gnost® T_4, RIA-gnost® FT_4 and RIA-gnost® FT_3, in which anti-T_4 or anti-T_3 antibody-coated tubes are used. The sensitivity of the kits was 2.5 ng/ml, 0.5 pg/ml and 0.6 pg/ml, respectively, with intra-assay coefficients of variation of 4.7, 5.5 and 5.0%, respectively. The plasma concentrations of total T_3 and rT_3 were determined using the double antibody method. The antibodies used in the analyses were Ab-T_3 (Sigma; working titre 1:6000), Ab-rT_3 (2/77; titre 1:8000), and the second antibody against rabbit immunoglobulins (Ab-II; working titre: 1:30). Anti-rT_3 antibodies were a gift from Prof. Jerzy Kosowicz (Medical Academy, Poznań), and the second ("antirabbit") antibody was obtained at the Department of Animal Physiology of the Agricultural University in Krakow after several immunizations of sheep with rabbit immunoglobulins. The analytical sample contained 100 µl of blood plasma (or plasma free of iodothyronine, in which T_3 or rT_3 standard was dissolved), 100 µl of isotope-labeled 125I-T_3 or 125I-rT_3 (spec. act. 135-165 mCi/mg; DuPont NEN, Belgium) and 100 µl of appropriate primary antibody. After 24 h incubation at room temperature, 100 µl Ab-II was added to each tube and the samples were incubated for 30 min. This was followed by adding 1 ml of 18% polyethylene glycol (PEG; Sigma). The samples were mixed and centrifuged for 30 min ($3000 \times g$). After centrifugation, the supernatant was removed and the samples were measured in a gamma counter (LKB, Finland). The sensitivities of these two methods were 0.05 and 0.03 ng/ml, with intra-assay coefficients of variation of 6.1 and 4.5%, respectively.

Statistical analysis

The results were analysed statistically by two-way analysis of variance followed by Tukey's test using the Statgraphics Plus 4.0 computer program. The results were considered significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

The effect of CLA supplementation on the level of blood lipid indicators in pigs is shown in Table 3. The use of CLA at 0.5-2.0% of the ration did not cause statistically significant changes in the blood levels of individual lipid fractions. However, the obtained data indicate a tendency towards increased concentrations of TG, TC and LDL cholesterol in the blood of CLA-supplemented pigs. Analysis of the effect of sex on these parameters studied showed that in barrows, plasma

Table 3. Concentrations of lipid indicators in blood plasma of fatteners obtaining conjugated linoleic acid (CLA) in diet, mg/dl

Indicator	% of CLA addition to the diet n=10 in each group					SEM	Sex		SEM	Interaction diet × sex
	0	0.5	1.0	1.5	2.0		gilts	barrows		P value
Triglycerides	43.64	45.30	47.78	40.33	39.49	3.60	39.8a	46.7b	2.27	0.16
Total cholesterol	75.61	87.17	84.64	81.81	81.55	3.34	80.7	83.5	2.11	0.33
HDL	32.17	32.49	31.40	31.63	31.75	1.49	32.4	31.3	0.94	0.03
LDL	34.71	45.61	43.68	42.11	41.90	2.83	40.3	42.8	1.73	0.81

[a,b] - P<0.05; SEM - standard error of mean

TG levels were significantly higher (P<0.05), with only an upward tendency for TC and LDL cholesterol.

Changes in the composition of fatty acids in the blood plasma of fatteners receiving different CLA rations are shown in Table 4. The dietary CLA supplement of 0.5-2.0% caused a gradual increase in the CLA fraction in blood from 0.37 to 3.14% (P<0.01). CLA had an effect on a significant (P<0.01) increase in the level of SFA, especially stearic acid (C 18:0; 12.5→14.1%). Dietary CLA supplementation caused a significant (P<0.01) decrease in the level of MUFA, particularly oleic acid (C 18:1; 24.2→15.3%) and PUFA, particularly n-6 PUFA, including linoleic acid (C 18:2; 32.2→27.4%) and arachidonic acid (C 20:4; 8.3→5.6%), while significantly increasing the level of n-3 UFA, timnodonic acid (C 20:5; 0.15→0.27%) and C 22:5 (1.04→1.31%). The increase in the dietary CLA level significantly lowered the n-6 PUFA to n-3 PUFA ratio in blood plasma from 18.2 to 13.2 (P<0.01). CLA also caused a significant (P<0.01) decrease in the Δ9-desaturase index from 0.48 in the control group to 0.36 in the group receiving 2% dietary CLA. Two-way ANOVA revealed significant interaction between the CLA-enriched diet and sex of the experimental animals, especially in the case of SFA (P<0.01), MUFA (P<0.02), PUFA *n-6/n-3* (P<0.01) and the Δ9-desaturase index (P<0.04) (Table 4). In comparison with control group values, only the 0.5% dietary CLA supplement significantly (P<0.01) increased the blood concentration of T_4 and T_3 in pigs: by 64 and 27%, respectively (Table 5). The statistical analysis showed that the effect of CLA on T_4 and T_3 concentrations in blood depended on the sex of fatteners. Moreover, the analysis revealed that the T_3 concentration in the plasma of barrows was significantly higher (P<0.05; Table 5). No significant effect of CLA on rT_3, FT_4 and FT_3 concentrations in the blood of the animals studied was found.

Table 4. Composition of fatty acids in blood plasma of pigs obtaining in diet different addition of conjugated linoleic acids (CLA), %

Fatty acids	% of CLA addition to the diet n=10 in each group					SEM	Sex		SEM	Interaction diet × sex
	0	0.5	1.0	1.5	2.0		gilts	barrows		
C 14:0	0.39aA	0.52bB	0.48abAB	0.41aAB	0.48abAB	0.02	0.46	0.45	0.01	0.02
C 14:1	0.07	0.06	0.07	0.06	0.08	0.02	0.08B	0.06A	0.01	0.25
C 15:0	0.21ab	0.24b	0.20ab	0.18a	0.22ab	0.02	0.22	0.20	0.01	0.54
C 16:0	14.56aA	16.74bB	17.39bB	16.51bB	16.82bB	0.21	16.28	16.52	0.13	0.01
C 16:1 *n-7*	1.36ab	1.58b	1.47ab	1.28ab	1.20a	0.09	1.39	1.37	0.05	0.20
C 17:0	0.66	0.75	0.66	0.59	0.71	0.05	0.66	0.69	0.03	0.68
C 17:1	0.32bAB	0.34bB	0.26ab	0.24ab	0.21aA	0.02	0.28	0.27	0.01	0.07
C 18:0	12.46aA	13.15abAB	14.08bB	14.07bB	13.56abAB	0.30	13.31	13.62	0.19	0.38
C 18:1 *n-9*	24.22eE	22.00dD	19.41cC	17.19bB	15.53aA	0.28	18.71A	20.63B	0.18	0.11
C 18:2 *n-6*	32.25bB	28.71aA	27.37aA	29.62aAB	28.82Aa	0.64	29.52	29.19	0.40	0.75
C γ18:3 *n-6*	0.39bB	0.37bB	0.34bAB	0.32abAB	0.23aA	0.02	0.33	0.33	0.01	0.57
C α18:3 *n-3*	0.57	0.61	0.54	0.53	0.55	0.03	0.58	0.54	0.02	0.80
CLA	0.37aA	1.22bB	1.68cBC	2.16dC	3.14eD	0.10	1.75	1.67	0.06	0.41
C 20:0	0.11aA	0.16abAB	0.18bcABC	0.21bcBC	0.25cC	0.02	0.17	0.20	0.10	0.36
C 20:1 *n-?*	0.35aA	0.45aA	0.64abAB	0.99bcBC	1.35cC	0.09	0.63	0.88	0.06	0.01
C 20:2 *n-3*	0.35	0.29	0.29	0.32	0.35	0.09	0.31	0.32	0.06	0.19
C 20:3 *n-6*	0.63	0.49	0.52	0.60	0.62	0.04	0.59	0.55	0.02	0.59
C 20:4 *n-6*	8.31cB	7.71bcB	6.48abAB	6.76abcAB	5.59aA	0.42	6.89	7.05	0.27	0.81
C 20:5 *n-3*	0.15aA	0.22abAB	0.20abAB	0.26bB	0.27bB	0.02	0.20	0.23	0.01	0.01
C 22:4 *n-6*	0.73ab	0.80b	0.71ab	0.73ab	0.58a	0.04	0.64A	0.78B	0.02	0.007
C 22:5 *n-3*	1.04a	1.28ab	1.23ab	1.31b	1.25ab	0.06	1.22	1.23	0.03	0.35
C 22:6 *n-3*	0.21	0.23	0.26	0.31	0.33	0.02	0.21A	0.33B	0.01	0.004
SFA	28.19a	31.32b	32.81c	33.71bc	33.92bc	0.35	32.89	31.49	0.22	0.01
MUFA	26.34eD	24.45dC	23.87cB	21.78bA	20.38aA	0.31	21.10A	23.23B	0.19	0.02
PUFA	45.03cC	43.97bBC	42.65aA	43.77bBC	43.20bAB	0.49	43.30	43.25	0.31	0.37
PUFA *n-6*/PUFA *n-3*	18.20bB	14.70aA	14.20aA	14.00aA	13.20aA	0.48	15.00	14.50	0.30	0.01
Δ9 desaturase index 1	0.48dD	0.44cC	0.40bB	0.38abAB	0.36aA	0.01	0.40A	0.42B	0.01	0.04

1- [MUFA/(SFA + MUFA)]; SEM - standard error of mean

^{a,b,c} - $P<0.05$; ^{A,B,C,D,E} - $P<0.01$

Table 5. Effect of different CLA addition in the fatteners' diet on thyroid hormone concentrations in blood plasma (n=10)

Item	% of CLA addition to the diet n=10 in each group					SEM	Sex		SEM	Interaction diet × sex
	0	0.5	1.0	1.5	2.0		gilts	barrows		P value
T_4, ng/ml	18.86A	30.98B	16.72A	16.62A	23.86AB	1.87	20.05	22.77	1.18	0.005
T_3, ng/ml	1.00abAB	1.27bB	0.86aA	1.22bAB	0.97abAB	0.07	0.99a	1.14b	0.04	0.02
rT_3, ng/ml	0.45	0.46	0.51	0.49	0.46	0.02	0.45	0.50	0.01	0.08
FT_4, pg/ml	2.33	13.46	12.60	13.27	14.22	0.80	12.72	13.63	0.50	0.26
FT_3, pg/ml	3.16	3.21	3.23	3.15	3.17	0.19	3.11	3.25	0.12	0.29

a,b - P<0.05; A,B - P<0.01; SEM-standard error of mean

DISCUSSION

Supplementing CLA is an effective method of changing the composition of fatty acids and lipids in pig blood and tissues (Ostrowska et al., 2003). In the present study, the CLA preparation increased the level of saturated acids, mainly C 18:0. These results concur with earlier studies that found a positive effect of CLA on the saturation of fatty acids in pig lipids (Migdał et al., 2004). The dietary CLA supplement was found to increase the level of n-3 UFA and to reduce the level of n-6 acids (Smith et al., 2002). A similar effect was observed in the present study, i.e. an increase in the level of n-3 C 20:5 and C 22:5 acids and a decrease in the level of n-6 acids, particularly C18:2 and C 20:4. Bani and Martin (1998) suggested that CLA selectively affects the elongation of n-3 acids and increases the degradation of n-6 acids. In studies with rats, CLA was shown to protect n-3 acids and to increase the utilization of n-6 acids (Li and Watkins, 1998). Changes in the level of C 18:3 and C 18:2 acids of the n-3 and n-6 series influence desaturase activity and thus lead to oxidation of double bonds and elongation of the carbon chain. The relationships between n-6 and n-3 PUFA are illustrated by their mutual proportions (n-6 PUFA/n-3 PUFA), the decrease of which is inversely proportional to the amount of CLA in the diet. A similar relationship between the amount of dietary CLA and the proportion of UFA in blood was shown by Ostrowska et al. (2003). In the present study we observed a diet-dependent effect of CLA on reducing the blood level of MUFA. A similar effect of CLA on the blood level of MUFA, which can be attributed to a gradual decrease in stearoyl-CoA desaturase activity, was noted in previous feeding trials (Smith et al., 2002). This suggestion is confirmed by a gradual decrease in the Δ9-desaturase index in animals receiving CLA (Table 4). The direct replacement of fatty acids with dietary CLA and its effect on desaturase and lipase activity are probably the main reasons for the changes observed in the fatty acid profile of blood plasma. In earlier studies, CLA was viewed as a hypocholesterolaemic factor that reduces atherosclerosis in rodents, mainly rats and rabbits (Corino et al., 2002).

In the present study, total cholesterol and LDL fractions in plasma of Polish Large White breed fatteners were similar to those found in the Pietrain breed, but were by about 20% lower in comparison with Duroc and Hampshire pigs of the same age (Migdał et al., 2003). CLA had no significant effect on the concentration of particular lipoprotein fractions, although there was a tendency towards increased levels of triglycerides, total cholesterol and HDL cholesterol. In pigs, similar changes in the blood lipid levels were observed after the administration of 1.65% CLA in the diet by Stangl et al. (1999). Munday et al. (1999) found aortic fatty streaks in mice fed CLA. In contrast, Sugano et al. (1997) found no differences in the concentration of HDL and total cholesterol in rats fed CLA at 1% of the ration. Based on the above results it is difficult to compare the quality and extent of changes in the lipoprotein profile with those reported in other studies, because lipid indicators vary according to not only the experimental diet but also the species and sex of the animals studied. Most of the above-mentioned studies investigated the effects of mixtures of a large number of CLA isomers, however, several lines of evidence indicate that the effect of CLA on plasma lipid profiles may depend on the effects of individual CLA isomers or the proportion between *c*9*t*11 and *t*10*c*12 CLA isomers in a diet. For instance, Tricon et al. (2004) showed the hyperlipidaemic effect of *t*10*c*12 CLA and hypolipaemic properties of *c*9*t*11 CLA isomers in blood plasma of healthy humans. A similar hyperlipidaemic effect following treatment with *t*10*c*12-CLA diet was found in mice (Arbones-Mainar et al., 2006). Further investigations are needed to explain the effect of individual CLA isomers on lipid profiles in the blood plasma of growing pigs.

In the present study we found a significant increase in the plasma concentrations of T_4 and T_3 in pigs receiving the lowest CLA dose (0.5%). Where higher CLA doses were given, changes in the blood levels of T_4 and T_3 were not statistically significant in relation to control group values. CLA had no effect on the levels of FT_4, FT_3 or rT_3. The level of T_3 was significantly higher in the blood of barrows than gilts, which may be related to the higher basal metabolic rate in the males (Danforth and Burger, 1989). Sawosz et al. (2005) reported that the blood concentration of iodothyronines in pigs is correlated with the level of dietary energy. Changes in the blood level of T_4 reflect thyroid activity and T_4 utilization in peripheral tissues (mainly in the liver and kidneys). Meanwhile, changes in the T_3 level are caused by both the activity of the thyroid (from which approximately 30% of this hormone originates) and the activity of D1 and D3 deiodinases, which are responsible for the conversion of T_4 into T_3 and rT_3 in peripheral tissues and then into 3,3'-T_2 (Wassen et al., 2004). It can therefore be suggested that the increased blood concentration of T_4 and T_3 in pigs fed CLA is related to a direct effect of CLA on the activity of the hypothalamic-pituitary-thyroid axis and/or deiodinase activity. On the other hand, the lack of significant changes in the level of free iodothyronines (i.e. FT_4 and FT_3) suggests that despite the effect of CLA (at low concentrations) on the blood level of total T_4 and T_3, these changes are

physiologically unimportant because body cells can be normally supplied with both free iodothyronines.

CONCLUSIONS

The supplementation of fatteners with dietary CLA alters mainly the profile of fatty acids in blood but has no significant effect on the levels of triglycerides, total cholesterol and cholesterol fractions. The lack of significant changes in the concentration of free thyroxine and triiodothyronine indicates that CLA supplementation may not significantly change the body's thyroid homeostasis.

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