

Dietary conjugated linoleic acid influences the content of stearinic acid in porcine adipose tissue

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ABSTRACT: The present study was conducted in order to determine the effects of supplementation of a growing-finishing pig diet with 0.5% conjugated linoleic acid (CLA) on production characteristics and slaughter traits. Ninety-seven female Swedish Landrace pigs were used. The control group of animals was fed a regular diet ($n = 49$), while the experimental group of animals ($n = 48$) received a diet where part of the soybean oil was substituted with commercially enriched CLA oil (containing at least 56% of CLA isomers, 28% *cis*-9, *trans*-11 and 28% *trans*-10, *cis*-12). The experiment lasted 44 days; porkers were fed from an initial weight of 66.0 up until a final weight of 103.5 kg. Feed conversion ratio, carcass and ham weight, percentage of lean meat and subcutaneous fat tissue as well as intramuscular fat were recorded. The fatty acid content of ham intramuscular fat tissue was determined by HPLC. No statistically significant influence of CLA was observed, either on carcass and ham weight, or on fat percentage in subcutaneous and intramuscular tissue. Dietary CLA enrichment proved to increase the content of stearinic acid in intramuscular fat tissue, 17.29 ± 13.26 % in experimental and 15.87 ± 33.71 % in control group of pigs ($P < 0.01$). The obtained production results show no statistically significant changes in main production traits between the two groups of animals. The observed difference in the content of stearinic acid ($P < 0.01$) implies firmer fat tissue, which has a practical value in pig bacon fattening.

Keywords: swine; conjugated linoleic acid; fat tissue; lean meat

Conjugated linoleic acid (CLA) is a fatty acid that has drawn significant attention in the last two decades for its potential health benefits. CLA is naturally found in milk and dairy products (Chin et al. 1992), and was originally identified as an anti-cancer component from ground beef extract (Pariza and Hargraves 1985; Ha et al. 1987). The strong interest of scientists in CLA can be attributed to the fact that it shown beneficial effects on the health of experimental (mice, rats, rabbits), domestic (swine and cattle), companion animals (dogs) and humans. Besides the ability to reduce body fat and increase non-fat (muscle) tissue, it has been discovered that conjugated linoleic acid also has anti-carcinogenic properties and reduce the incidence of arteriosclerosis (Kelley et al. 2007).

Although there are a number of CLA isomers, the primary natural isomer of CLA in food is the *cis*-9, *trans*-11 isomer (Chin et al. 1992; Kramer et al. 1998). The *cis*-9, *trans*-11 CLA isomer in food can be derived from one of two pathways: from the incomplete biohydrogenation of linoleic acid to stearic acid by rumen bacteria or from the *delta*-9 desaturation of *trans*-11 vaccenic acid (a primary intermediate for ruminant biohydrogenation) in mammalian tissues (Kay et al. 2004). This isomer comprises up to 80–85% of total CLA in food (Chin et al. 1992). Since CLA is also available as a food additive, it can be directly added to foods. Most current CLA research using synthetically prepared CLA (consisting mainly of two isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12) has shown a

Supported by the Ministry of Science and Technology of the Republic of Serbia (Grant No. III 46002).

number of biological benefits, such as prevention of cancer, atherosclerosis, obesity, diabetes and osteoporosis (Park and Pariza 2007). Not all of these conditions have been positively correlated with the naturally occurring *cis*-9, *trans*-11 CLA. For example, CLA is associated with decreased body weight. However, this bioactivity is primarily associated with the *trans*-10, *cis*-12 CLA isomer (Park and Pariza 2007). The most significant bioactivity of the *cis*-9, *trans*-11 CLA isomer is its anti-carcinogenic property, as shown in a number of animal cancer models such as breast, epidermis, prostate, colon, liver, kidney, and lung (Brown et al. 2004; Kelley et al. 2007). There are limited studies about the effects of naturally occurring CLA on human cancer incidence. Knekt et al. (1996) reported an inverse relationship between milk intake and incidence of breast cancer which might be associated with CLA content in milk. This observation is confirmed by Aro et al. (2000), in postmenopausal women. However, others reported weak or no association between milk and daily consumption and CLA levels and breast cancer in humans (Chajes et al. 2002; Larsson et al. 2009). Overall, it is not conclusive if naturally occurring CLA has a significant health impact on prevention of cancer. Thus, further studies are needed to confirm the implications of CLA on human cancer prevention.

Since CLA is primarily found in fat tissue, CLA concentrations are highly influenced by dietary fat concentrations. Muscle CLA concentrations can also be increased by the direct inclusion of CLA into animal feeds which has the additional benefit of decreasing muscle adipose fat percentage (Dugan et al. 1997). CLA concentrations in beef can be influenced by diets containing oils or oilseeds high in polyunsaturated fatty acids (usually linoleic or linolenic). Dietary practices can increase CLA concentrations up to three fold (Madron et al. 2002). Ostrowska et al. (1999) evaluated the use of CLA in pigs and found that increased levels of CLA in feed induced a significant decrease in daily fat accretion rate. On the other hand, water and protein accretion was increased in group of pigs supplemented with CLA.

The hypothesis tested in this study was that a commercial CLA preparation (Lutalin[®], BASF) at a constant level (0.50%) in the growing-finishing diet would improve feed efficiency and decrease carcass subcutaneous fat tissue. Thus, our objective was to determine if the CLA feed supplementation in growing-finishing pigs could function to increase

lean meat percentage, intramuscular fat tissue and serve to decrease subcutaneous fat tissue. Also, the aim of the trial was to determine if any difference was elicited in the quality of meat (with regard to chemical composition and major production traits) and fat tissue (with regard to its content and quantity of fatty acids).

MATERIAL AND METHODS

The animal care and experimental procedures used in this experiment were in accordance with European Community guidelines (Directive 86/609/EEC) and approved by the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade.

According to the experimental design animals were allocated into one of two groups: the control group ($n = 49$ pigs) or the experimental group ($n = 48$ pigs).

Female pigs (Swedish Landrace) were of approximately equal body mass (66 ± 3.0) kg and age (approximately 130 days). The trial lasted 44 days. Before the trial commenced the health status of all animals was determined and this was later monitored throughout the entire trial period. The pigs were randomly allotted into one of two treatment groups. Animals in the two groups were fed a diet of the same chemical composition. The only difference in diet composition between these two groups of pigs was that the experimental group were fed meal in which one part of soybean oil was substituted with a commercial CLA oil preparation (final concentration of 0.5% in feed). The ingredients and chemical composition of the experimental diets are shown in Table 1, while the main fatty acids and analysis of soybean oil is described in Table 2. The commercial CLA oil preparation used in this trial (Lutalin[®], BASF Corporation, NJ, USA) contained a minimum 56% of conjugated linoleic acid (C18:2) in ester form (28% *cis*-9, *trans*-11 and 28% *trans*-10, *cis*-12 isomers) in an amount of 0.5%; thus, each animal in the experimental group received approximately 15 g Lutalin[®]/day, or over the course of the trial period (44 days) every pig consumed approximately 660 g of Lutalin[®]. Both diets were formulated to provide similar protein and energy levels, fulfilling the nutritional needs for female pigs at considered weights by the NRC (1998). The pigs were housed in an environmentally controlled experimental grower/finisher shed.

Table 1. Raw and chemical composition of pig feed for the control and experimental groups

	Group	
	control	experimental
Ingredient (%)		
Corn	68.75	68.75
Wheat bran	10.00	10.00
Soybean meal (44% CP)	14.90	14.90
Sunflower meal (33% CP)	2.00	2.00
Soybean oil	1.25	0.75
CLA oil*	–	0.50
Limestone	1.30	1.30
Monocalcium phosphate	0.80	0.80
NaCl	0.45	0.45
Vitamin and mineral premix**	0.50	0.50
Synthetic lysine	0.05	0.05
Chemical composition (%)		
Crude fibre	3.26	3.26
Crude protein (CP)	14.29	14.29
Crude fat	4.52	4.52
Ash	4.73	4.73
Metabolic energy (calculative) MJ/kg	13.80	13.80

*commercial CLA oil preparation – Lutalin®, BASF Corporation NJ, USA

**vitamin and mineral premix – provided the following nutrients per kg of air dry complete feed (mg): retinol 6.4, cholecalciferole 0.083, α -tocopherol 22, menadione 0.60, riboflavine 3.3, nicotinic acid 16.5, pantothenic acid 5.5, pyridoxine 1.1, biotin 0.56, choline 11 000, cyanocobalamine 0.017, Fe 88, Zn 55, Mn 22, C 6.6, I 0.22, Se 0.1

They were group-housed (12 pigs per pen) and had *ad libitum* access to feed (single space dry feeders) and fresh water (nipple drinkers) until the end of trial. Before the beginning of the experiment all pigs were subjected to the same feeding and management. Pigs were sacrificed at a local abattoir at the average (103.5 ± 3.8) kg live weight.

The determination of the composition of feeds (in triplicate) was carried out according to the recommendations of the AOAC (2005). Protein content was estimated using the Kjeldahl method. Total fat was determined as diethyl ether extract after Soxhlet extraction. Ash was determined by burning oven-dried samples in a muffle furnace at 600 °C. Total lipids were extracted from samples of feed (soybean oil) and tissue with chloroform/methanol (2 : 1, v/v) using the method of Folch et al. (1957). Fatty acid methyl esters (FAMES) were prepared by reacting the chloroform/methanol extract with sodium methoxide (0.5M) in methanol (Sigma–Aldrich, Germany) for 15 min at 50 °C (Kramer

1998). Pentadecanoic acid (C15:0) (Sigma–Aldrich, Germany) was used as an internal standard. Fatty acid methyl esters (FAMES) were analysed using the Hewlett–Packard HP-6890 (Avondale, PA, USA) gas chromatograph equipped with a flame ionization detector and a capillary column HP-Innowax (30 m \times 0.32 mm *i.d.* and 0.25 μ m polyethylene glycol-film thickness; Lopez-Bote et al. 2003). For quantification, the response factor was calculated and the area normalization method used. The results were reported as the weight percentage of total FAMES.

At slaughter, body weight, carcass weight and carcass yield were recorded. In addition, on the warm carcass, the thickness of subcutaneous fat tissue with skin was measured in order to calculate the meat percentage by the method of two points. Samples for analyses were vacuum-packed in low-oxygen permeable film, and kept frozen at –20 °C prior to fatty acid analysis. Analyses were carried out within four weeks after slaughtering. Carcass

Table 2. Fatty acid composition of soybean oil used in the feed mixture

Iodine value (g/100g)	132
Saponification value (mg KOH/g)	191
Fatty acids composition (%)	
C14:0	traces
C16:0	10.83
C18:0	4.48
C18:1	23.59
C18:2	53.30
C18:3	7.42
C20:0	0.37
Saturated fatty acids (%)	15.58
Mono unsaturated fatty acids (%)	23.59
Poly unsaturated fatty acids (%)	60.72

sides were then placed into a cooling chamber where they remained for an average of around 20 h for cooling (until a temperature of +2 to +4 °C was achieved). Subsequently, the ham was separated from the carcass, by a cut from the last loin and first rump vertebrae, and a second thigh was cut through the knee joint (*articulatio genus*). By dissection of the ham, muscle, subcutaneous fat and intramuscular fat tissues, as well as skin and bones were separated. Weight measurements were done on scale of ± 5 g accuracy. Calculation of lean muscle percentage in pig carcass was based on thickness (mm) of subcutaneous fat tissue with skin, measured on warm carcasses between the third and fourth lumbar vertebrae (X_1) and the thickness of subcutaneous fat tissue on the back, measured in the centre of the back, between the third and fourth rib (X_2), according to the following formula: Lean meat (%) = $67.098 - (0.505X_1 + 0.14X_2)$ (NSIF 1997). The quantity (kg) of subcutaneous fat tissue was determined by dissection of the ham. The quantity

of intramuscular fat was determined by extraction (Ha et al. 1987). The quantity of saturated (stearine) and unsaturated (linoleic) fatty acids was determined by gas chromatography (from intramuscular fat extracted from the ham) as described for dietary fatty acids. Iodine and saponification values were determined according to the procedure described in the Code of Regulations Standard SRPS ISO 3961:2001 in the belly fat tissue extracted using the method of Folch et al. (1957).

Statistical processing of data was carried out using a method of variance analysis (ANOVA) according to Snedecor and Cochran (1971) and significance of differences was determined with the Tuckey LSD test.

RESULTS

Over the course of the trial no significant health disorders were observed in either the control or experimental group of animals. Both diets completely satisfied the nutritional needs of the growing-finishing pigs.

The reduction results obtained in this trial are shown in Table 3. No significant changes in total feed consumed were observed between the control and experimental group of pigs. Body mass gain was higher in the group of pigs fed the diet supplemented with CLA (38.25 ± 4.98 kg) compared to the control group of pigs (37.14 ± 5.40 kg). The group of animals fed the diet supplemented with CLA had a better feed conversion ratio (3.290) compared to the control group of animals fed a standard diet (3.401); expressed in percentage feed conversion ratio the improvement elicited by CLA supplementation was 3.26%.

The slaughter characteristics of both groups of pigs are shown in Table 4. The determined carcass weights were not significantly different between the

Table 3. Production results of the control and experimental groups of pigs

Parameter	Group	
	control ($n = 49$)	experimental ($n = 48$)
Initial weight (kg)	66.18 ± 3.44	65.44 ± 3.11
Average gain (kg)	37.14 ± 5.40	38.25 ± 4.98
Average feeding period (days)	44.25	44.71
Total feed consumed – as fed (kg)	126.33	125.83
Feed conversion ratio	3.401	3.290
Average daily gain (kg)	0.839	0.855

Table 4. Slaughter characteristics of both groups of pigs

Parameter	Group				P ₁ –P ₂
	control (<i>n</i> = 49)		experimental (<i>n</i> = 48)		
	$\bar{x} \pm \text{SD}$	CV (%)	$\bar{x} \pm \text{SD}$	CV (%)	
Slaughter weight (kg)	103.53 ± 3.79	3.66	103.69 ± 3.79	3.66	0.16 ^{NS}
Carcass weight (kg)	80.35 ± 4.32	5.38	80.39 ± 3.34	4.15	0.04 ^{NS}
Loin fat thickness (mm)	27.02 ± 5.51	20.38	25.63 ± 5.28	20.61	1.39 ^{NS}
Back fat thickness (mm)	17.37 ± 3.79	21.82	16.94 ± 3.80	23.75	0.43 ^{NS}
Muscle tissue in carcass (%)	51.02 ± 3.19	6.25	51.79 ± 3.06	5.91	0.77 ^{NS}
Ham weight (kg)	9.277	–	9.356	–	–
Ham subcutaneous fat tissue (%)	14.81 ± 4.11	27.74	14.13 ± 3.42	24.18	0.68 ^{NS}
Ham intramuscular fat tissue (%)	5.32 ± 1.19	22.36	6.03 ± 1.19	19.87	0.71 ^{NS}
Intramuscular fat in <i>m. gluteus</i> (%)	1.64 ± 0.64	39.48	1.84 ± 0.69	37.40	0.202 ^{NS}

P₁ = average values of control group, P₂ = average values of trial group, NS = statistically not significant

groups. By calculating the percentage of lean meat in each carcass it was determined that the group of animals fed the diet supplemented with 0.5% CLA had a higher percentage (51.79 ± 3.06) than did the group of animals fed standard diet (51.02 ± 3.19), but this difference was not statistically significant. The determined average percentage of subcutaneous fat tissue in ham in our trial was lower in the group of pigs fed the diet supplemented with 0.5% CLA (14.13 ± 3.42%) than in the control group of pigs (14.81 ± 4.11%). Besides that, loin fat thickness in the experimental group (25.63 ± 5.28 mm) as well as back fat thickness (16.94 ± 3.80 mm) were lower than in the control group of pigs (27.02 ± 5.51 mm) for loin and (17.37 ± 3.79 mm) for back fat tissue. However, these results were not statistically significant. The percentage of intramuscular fat tissue determined in ham was 6.03 ± 1.19% in experimental and 5.32 ± 1.19% in the control group

of animals, in gluteus muscle fat tissue percentage was also higher in the experimental (1.84 ± 0.69%) than in the control group of pigs (1.64 ± 0.64%). The average ham weight of the group of pigs fed a diet supplemented with CLA was higher (9.356 kg) than the weight of ham in the control group of swine (9.277 kg). However, the above mentioned differences were not statistically significant.

Fatty acid content is shown in Table 5. A higher content of stearinic acid in intramuscular fat tissue in the group of animals fed a diet supplemented with CLA (17.29 ± 13.26%) in comparison to intramuscular fat tissue in the control group of animals (15.87 ± 33.71%) was determined. This difference was statistically significant ($P < 0.01$). A similar situation was observed for linoleic acid (15.13 ± 19.44% in control and 14.84 ± 46.75% in experimental group of animals) but this difference was not statistically significant. The same trend was

Table 5. Proportions of stearine and linoleic acid and iodine extracted from ham intramuscular fat tissue

Parameter	Group				P ₁ –P ₂
	control (n = 49)		experimental (n = 48)		
	$\bar{x} \pm \text{SD}$	CV (%)	$\bar{x} \pm \text{SD}$	CV (%)	
Stearine acid in intramuscular fat (%)	15.87 ± 33.71	21.24	17.29 ± 13.26	7.67	1.418**
Linoleic acid in intramuscular fat (%)	14.84 ± 46.75	31.51	15.13 ± 19.44	12.85	0.290 ^{NS}
Stearine to linoleic acid ratio	1.09 ± 0.38	35.17	1.16 ± 0.19	16.01	–
Iodine value (belly fat tissue)	63.23 ± 36.43	5.76	64.03 ± 32.30	5.06	0.805 ^{NS}

P₁ = average values of control group, P₂ = average values of trial group, NS = statistically not significant

**statistically significant ($P < 0.01$)

observed for the stearine to linoleic acid ratio, which was 1.164 for the intramuscular fat tissue of animals fed the diet supplemented with CLA and 1.091 for the intramuscular fat tissue of animals fed a regular diet. The iodine value of belly fat tissue in the control group of pigs was 63.23 ± 36.43 and in experimental group of pigs 64.03 ± 32.30 , but this difference was not statistically significant.

DISCUSSION

Although the recorded differences for feed conversion between the two groups were not statistically significant, the U.S. pork industry claims to achieve a feed conversion ratio of 3.4 to 3.6. Therefore, the values recorded in this trial speak in favour of CLA feed supplementation. These results are in compliance with the results of Ostrowska et al. (1999), who reported an increase in feed conversion ratio when supplementing with CLA but with the most pronounced difference in the first four weeks of the treatment. They concluded that the supplementation of diet with CLA increased the feed conversion ratio by 6.3%. In our trial no statistically significant difference in average daily gain between the control and experimental group was found. However, Su et al. (2006) reported a significantly higher average daily gain ($P < 0.01$) in pigs fed a diet supplemented with 3.0 g/kg of CLA. On the other hand, Thiel et al. (1998) reported linear increases for average daily gain and feed conversion ratio with increasing levels of CLA from 0.12% to 1.0% in the diet, but it should be mentioned that these experiments were conducted on crossbreeds.

Carcass weights were not affected by dietary CLA supplementation, which is in agreement with previous reports (Park et al. 1997; Wiegand et al. 2001).

The calculated percentage of lean meat in our study is widely in agreement with Pulkrabek et al. (2006). However, Wiegand et al. (2002) have found that dietary CLA did improve pig composition as measured by loin muscle area. Furthermore, these authors reported that percentages of lean meat increased linearly ($P < 0.01$) with increasing time on a CLA diet. In contrast, Cook et al. (1998) and Thiel et al. (1998) reported no differences in loin muscle cross-sectional area but did observe decreases in back fat thickness with increasing levels of CLA in the diet of pigs, whereas Dugan et al. (1997) reported increases in loin muscle mass.

We did not observe significant differences in back fat thickness. However, Cook et al. (1998), Ostrowska et al. (1999), Wiegand et al. (2001), and Su et al. (2006) reported that CLA supplementation results in decreases in subcutaneous fat tissue content. In our trial, besides a decrease in subcutaneous fat tissue, an increase in the percentage of intramuscular fat tissue in the group of pigs fed a diet supplemented with 0.5% CLA was noted.

Although the differences in ham weight in our study did not reach statistical significance, Wiegand et al. (2001) reported an increase in intramuscular fat as well as marbling in the *longissimus dorsi* of CLA-fed pigs. The findings of Dugan et al. (1997) and Cordero et al. (2010) were similar and they found a higher marbling score in pigs receiving diets enriched in CLA and slaughtered around 100 kg live weight. However, no effects of dietary CLA on IMF concentration have been found in pigs slaughtered at heavier weights (> 125 kg) (Lauridsen and Henckel 2005; Corino et al. 2006). Park et al. (1997) were the first to demonstrate that CLA modulates body composition. In their study, male and female mice given a 0.5% (wt/wt) CLA mixture had 57% and 60% lower body fat mass, respectively, than controls. The antio-besity mechanisms of CLA are complex and not fully understood. So far it was shown that CLA influences energy metabolism in mammals by decreasing energy intake or increasing energy expenditure. CLA has been proposed to reduce adiposity by elevating energy expenditure via an increased basal metabolic rate, thermogenesis or lipid oxidation in animals (Terpstra et al. 2002). CLA inhibits adipogenesis – the conversion of preadipocytes into adipocytes involves the activation of key transcription factors such as peroxisome proliferator-activated receptor γ (PPAR γ) and CAAT/enhancer binding protein (C/EBP). There is evidence that CLA suppresses preadipocyte differentiation in animals (Di Giancamillo et al. 2007; Miller et al. 2008) and humans (Brown et al. 2004) preadipocytes. CLA increases inflammation – although the primary function of white adipose tissue is energy storage, it also has the ability to produce a number of proinflammatory cytokines. These adipokines (i.e., cytokines produced by adipose tissue) can cause insulin resistance, thereby suppressing lipid synthesis and increasing lipolysis in adipocytes.

Ostrowska et al. (2003) reported that the percentage of palmitic acid (16:0), stearic acid (18:0), and palmitoleic acid (16:1) increased ($P < 0.05$) in

bacon tissues from CLA-fed pigs; these findings were similar to ours. Typically, *de novo* synthesis of saturated fatty acids (firmer fat) occurs in pigs (O'Hea and Leveille 1969). This is in complete accordance with our results, since a higher content of stearinic acid was determined in the group of pigs fed a diet supplemented with CLA. However, pigs with a genetic predisposition for less subcutaneous fat can also be expected to produce carcasses with more unsaturated fatty acids which was also the case in our trial (a higher content of linoleic acid in the experimental over the control group of swine; Correa et al. 2008). These changes toward more unsaturated fatty acids are presumably a function of less *de novo* fatty acid synthesis and greater uptake of dietary fatty acids. Moreover, the ability of CLA to modify fatty acid composition increases meat oxidative stability and, as a consequence, its shelf life. It was observed that dietary CLA supplementation in heavy pigs and rabbits results in improved muscle oxidative stability, as measured as ThioBarbituric Acid Reactive Substances (TBARS; Corino et al. 2008). Dietary CLA supplementation in heavy pig and rabbits significantly increased muscle CLA concentration in both meat and meat products (Pastorelli et al. 2005; Corino et al. 2008). The increase in CLA content suggests that the nutritional quality for human consumption may be improved and this is important in addition to the potential value-added marketing for health-aware consumers. Moreover, given the beneficial effects that CLA is acknowledged to have on human health, the possibility of increasing the CLA content in meat and cured ham appears to represent an opportunity to increase the health-promoting properties of these products.

It is noteworthy that CLA has the ability to improve the technological quality of meat and meat products which is related to its capacity to increase tissue saturation (Corino et al. 2006). The Parma Ham Production Consortium recommended in pig adipose tissue a maximum level of 70 for iodine, a measure of unsaturation, and therefore an indirect indicator of fat firmness, to avoid fat quality problems (Corino et al. 2006).

The obtained production results show no statistically significant changes in the main production traits between the two groups of animals.

The observed differences in the content of stearinic acid ($P < 0.01$) will result in fat tissue that is firmer which has practical value in pig bacon fattening.

Further research should be carried out to identify the mechanistic control of CLA responses in growing-finishing pigs and to determine the effect of CLA-containing foods on human health.

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Received: 2011–07–09

Accepted after corrections: 2012–02–13

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