Influence of dietary conjugated linoleic acid on growth, meat quality, lipogenesis, plasma leptin and physiological variables of lipid metabolism in rabbits[1,2]

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ABSTRACT: We investigated the effects of conjugated linoleic acid (CLA) supplementation on growth, feed efficiency, carcass characteristics, meat quality, lipogenesis, and lipid metabolism in rabbits. One hundred forty-four New Zealand White rabbits, half males, half females, age 55 d, mean 1.8 kg BW, were randomly assigned to three weight- and sex-balanced feeding groups in which conventional pelleted diets were supplemented with 0, 0.25, or 0.5% of a CLA preparation. The CLA preparation contained 65% CLA isomers. Twelve rabbits (six males and six females from each group) were slaughtered at each of three slaughtering trials (2.5, 2.8, and 3.1 kg BW, or 76, 90, and 104 d of age). Conjugated linoleic acid supplementation did not influence growth performance ($P \geq 0.05$) or carcass characteristics but reduced perirenal fat at heavier slaughtering weights ($P = 0.09$ at 2.8 kg BW; $P < 0.01$ at 3.1 kg BW). Conjugated linoleic acid reduced acetyl-CoA-carboxylase (CBX) activity in liver ($P < 0.05$) and adipose tissues ($P < 0.01$) but did not influence malic enzyme (ME) or glucose-6-phosphate dehydrogenase activity. Significant differences were found between sex in interscapular fat ($P < 0.05$) for CBX, in perirenal ($P < 0.01$) and interscapular ($P < 0.05$) fat for ME, and a tendency ($P = 0.070$) in liver for glucose-6-phosphate dehydrogenase. The oxidative stability of longissimus lumborum muscle was increased at the higher level of supplementation ($P < 0.05$). Conjugated linoleic acid reduced ($P < 0.05$) triglycerides and total cholesterol in plasma with a trend to increased serum leptin ($P = 0.06$). Plasma triglycerides were higher in males than females ($P < 0.01$) and plasma leptin tended to be higher in females (2.57 vs. 2.13 ng/ml, $P = 0.06$). It is concluded that dietary CLA reduced carcass fat in rabbits slaughtered at 2.8 kg or above and altered lipid metabolism to produce lower concentrations of serum triglycerides and total cholesterol and higher concentrations of leptin.

Key Words: Cholesterol, Conjugated Linoleic Acid, Leptin, Lipogenesis, Nutrition, Rabbits

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Introduction

Conjugated linoleic acid (**CLA**) is a mixture of geometrical and positional isomers of linoleic acid in which the two double bonds are conjugated. Numerous CLA isomers are possible, depending on the positions of the double-bond pairs (Lavillonnière et al., 1998; Sehat et al., 1998; Yurawecz et al., 1998). The configuration of the double bonds can also vary to increase the number of possible isomers: *cis-trans*, *trans-cis*, *cis-cis*, and *trans-trans* configurations are all possible.

The importance of CLA is not only that it has significant antioxidant and anti-obesity activity (Lin et al., 1995), but it also has anticarcinogenic activity in a variety of animal models (Ip et al., 1994; Belury, 1995; Banni and Martin, 1998). In addition, CLA has a protective effect against atherosclerosis in rabbits (Lee et al., 1994).

Recent research indicates that CLA can be useful as a growth-promoting nutritional supplement in pigs (Thiel-Cooper et al., 1998a; Eggert et al., 1999) and can decrease body fat content in mice (West et al., 1998) and pigs (Cook et al., 1998; Thiel-Cooper et al., 1998b;

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Sparks et al., 1999). Studies on the metabolic effects of CLA in intact animals and adipocyte cultures suggest that CLA directly affects key enzymes and processes involved in lipid mobilization and storage (Park et al., 1997).

However, studies of the effects of CLA on growth and meat quality in rabbits have not been published. The present study examined the effects of dietary supplement of CLA on growth, carcass characteristics, and meat quality in growing-finishing rabbits. We also investigated lipogenic enzyme activities in liver and adipose and plasma leptin and lipid profiles. To explore the development of fat deposits, animals were slaughtered at three different ages, 76, 90, and 104 d of age, corresponding to approximately 2.5, 2.8, and 3.1 kg BW, respectively.

Materials and Methods

Animals and Diets

The animals used in this experiment were cared for in accordance with the guidelines established by the European Community (no. 86/609/CEE) and approved by the Italian Ministry of Health (L. n. 116/92). One hundred forty-four New Zealand White rabbits, half males and half females, 55 d old, approximately 1.8 kg BW, were randomly assigned (equal proportions of males and females) to one of three diets. The animals were given ad libitum access to a commercial pellet diet (crude protein 16%, crude fiber 14%, ether extract 3%, and ash 8%, as fed) from Progeo s.r.l. Reggio Emila, Italy. The diet contained 0, 0.25, or 0.5% of a CLA preparation (Conlinco, Detroit Lakes, MN) in free fatty acid form. The diet met the nutritional requirement of rabbits of this age and strain (NRC, 1977). The CLA oil was synthesized from sunflower oil and contained approximately 65% of pure CLA isomers with 50% *cis*-9, *trans*-11 isomer and 50% *trans*-10, *cis*-12 isomer (from certificate of analysis provided by the manufacturer). The animals were housed two to a cage, paired by similar weights and the same sex. Body weights and feed intake were recorded on d 21, 35, and 49 of the trial.

Carcass Measurements and Collection of Adipose and Muscle

At the three slaughter ages (76, 90, and 104 d) 12 rabbits (six males and six females) of appropriate weight were selected from each dietary treatment. After slaughtering, the carcasses were left 24 h at 1°C ± 0.5°C, then carcass weight and interscapular and perirenal fat weights were recorded. On longissimus lumborum (**LL**) muscle, pH (Hama instruments Hi 9023 pH-meter) and L*, a*, and b* color values (Colorimeter and CR-300 camera, Minolta, Osaka, Japan) were also measured at that time.

At the second slaughtering only (age 90 d), a sample of LL muscle was taken 24 h after slaughtering from each animal and stored at –35°C pending determination of thiobarbituric acid-reactive substances (**TBARS**), a measure of the resistance of the muscle lipids to oxidation. Liver and perirenal and interscapular fat samples were also taken from each animal slaughtered at 90 d to determine lipogenic enzyme activity. These samples were frozen immediately in liquid N_2 and stored at –80°C pending analysis.

Measurement of Oxidative Stability of Muscle

The oxidative stability of rabbit muscle was determined using a modification of the method described by Monahan et al. (1992), which was a combination of the Kornbrust and Mavis (1980) method for the induction of lipid peroxidation and the Beuge and Aust (1978) method for determination of extent of lipid peroxidation by assaying 2-thiobarbituric acid-reactive substances (TBARS), as reported by Oriani et al. (2001). After 140 min an aliquot was removed for TBARS determination, expressed as nanomoles of malondialdehyde (**MDA**)/gram of muscle tissue per minute.

Enzyme Assays

To determine lipogenic enzyme activities in liver and adipose tissues, weighed quantities of tissue were homogenized in 0.25 *M* sucrose buffer and centrifuged at 30,000 × *g* for 40 min. The supernates were analyzed for malic enzyme (**ME**, EC 1.1.1.40) and glucose-6-phosphate dehydrogenase (**G6PDH**, EC 1.1.1.49) activities using a modification (Gandemer et al., 1983) of the methods of Hsu and Lardy (1969) and Fitch et al. (1959). The formation of NADPH was measured at 37°C by absorbance at 340 nm. Acetyl-CoA-carboxylase (**CBX**, EC 6.4.1.2) was assayed by the $H^{14}CO_3$-fixation method (Chang et al., 1967; Chakrabarty and Leiville, 1968, 1969). The activity of G6PDH and ME were expressed as micromoles of NADPH produced per total organ weight. Acetyl-CoA-carboxylase activity was expressed as nanomoles of bicarbonate incorporated per tissue.

Blood Sampling and Analysis

Blood samples were collected from all animals slaughtered at 90 d of age. Plasma was stored at –80°C pending analysis. Total cholesterol, triglycerides, and NEFA were determined in plasma by enzymatic spectrophotometric assay (Boehringer Mannheim, Germany). Serum leptin concentrations, expressed as nanograms per milliliter of human equivalent (**HE**), were determined with a commercially available radioimmunoassay procedure (Multi-Species Leptin RIA Kit, Linco Research, MO) using an antibody raised against human leptin. The cross-reactivity of this antibody has been tested against pig (67%), rat (61%), and mouse leptin (73%) (http://www.lincoresearch.com). The detection limit was 0.9 ng/mL. Intra-assay and interassay coefficient of variations were 3.7 and 7.8%, respectively. To test the sensitivity of the method, serial dilutions (25

Table 1. Growth performance of rabbits fed diets containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA)

Item	n	% Dietary CLA added			Sex		SEM
		0	0.25	0.5	Male	Female	
Body weight, g							
Age 55 d (d 0)	144	1,852.9	1,854.6	1,859.4	1,870.7	1,842.0	36.23
Age 76 d (d 21)	139	2,503.2	2,451.2	2,511.3	2,506.0	2,470.4	45.76
Age 90 d (d 35)	101	2,804.8	2,772.0	2,839.4	2,797.3	2,813.2	59.24
Age 104 d (d 49)	64	3,052.2	3,100.5	3,046.8	3,046.4	3,089.4	114.92
ADFI, g/d[a]							
Day 0 to 21	24	128.3	127.7	128.1	126.9	129.1	3.08
Day 21 to 35	18	126.3	122.4	125.0	124.6	124.6	2.91
Day 35 to 49	12	147.2	165.2	140.7	155.1	147.1	13.43
ADG, g/d							
Day 0 to 21	139	32.6	29.7	32.5	31.7	31.4	1.44
Day 21 to 35	101	22.1	25.3	22.9	21.9	25.0	2.21
Day 35 to 49	64	23.6	25.2	23.9	23.8	24.8	3.22
Gain/feed, g/kg[a]							
Day 0 to 21	24	254.1	232.6	253.7	249.8	243.2	6.76
Day 21 to 35	18	180.0	206.7	183.2	175.8	200.6	9.49
Day 35 to 49	12	160.3	152.5	169.8	153.8	168.3	15.20

[a]Feed intake was measured in one of every three cages for each dietary treatment.

to 100 μL) of rabbit plasma in buffer (200 μL final volume) were assayed and parallelism to the human leptin reference curve was detected ($P < 0.01$). Other studies on circulating rabbit leptin had used the same Multi-Species kit and showed that the antibody was sensitive enough to detect a 30% diurnal variation in plasma leptin (Rosi and Copalbo, 1999) and a 10 to 20% plasma leptin reduction following chronic treatment with clenbuterol (Corino et al., 1999).

Statistical Analysis

Statistical analysis was performed by factorial analysis of variance with SPSS (SPSS, Chicago, IL). Carcass weight was used as a covariate in the analysis of interscapular and perirenal fat weights. Comparison between means was by the Student-Neuman-Keuls *t*-test. The treatment × sex interaction was not significant and is not reported in the results.

Results

Growth Performance. No differences ($P \geq 0.05$) were observed among the three dietary groups for growth, feed intake, or feed efficiency at any slaughtering age (Table 1).

Carcass Characteristics and Meat Quality. Carcass data, pH, and color indices of LL muscle at ages of 76, 90, and 104 d are shown in Tables 2, 3, and 4, respectively. At the first slaughtering (76 d of age) the dressing percentage was higher ($P < 0.01$) in males than in females (Table 2). No other differences ($P \geq 0.05$) at first slaughtering were found. At the second slaughtering (90 d of age), perirenal fat tended ($P = 0.089$) to be lower in the treated groups than in controls (Table 3). No other differences at the second slaughter were found ($P \geq 0.05$). At the third slaughtering (104 d of age; Table 4), perirenal fat weight was lower in rabbits fed CLA ($P = 0.006$) and in males than in females ($P = 0.04$); no other differences ($P \geq 0.05$) were found at this slaughtering.

Mean basal TBARS values (nmol MDA/g tissue ± SEM) were 14.70 ± 0.79 for 0% CLA, 15.91 ± 0.82 for 0.25% CLA, and 16.96 ± 1.16 for 0.5% CLA. Mean TBARS after 140 min were 29.84 ± 2.18 for 0% CLA, 26.74 ± 2.28 for 0.25% CLA, and 25.24 ± 2.26 for 0.5% CLA with the following increments at 140 min compared to basal: 203% for 0% CLA, 168% for 0.25% CLA, and 149% for 0.5% CLA.

As shown in Figure 1, the rate of TBARS production (and hence lipid peroxidation) decreased as dietary supplementation of CLA increased, from 0.11 ± 0.01 nmol MDA·g tissue^{-1}·min^{-1} in the 0% CLA group to 0.059 ± 0.01 nmol MDA·g^{-1}·min^{-1} in the 0.5% CLA group. However, only the rate of TBARS production in the 0.5% CLA group was significantly lower ($P < 0.05$) than that in the 0% CLA group.

Enzyme Activities. The activities of CBX, ME, and G6PDH are shown in Table 5. No differences ($P \geq 0.05$) were observed among the three dietary groups for G6PDH and ME activities ($P \geq 0.05$), but 0.5% CLA supplementation reduced CBX activity in perirenal and interscapular fat ($P > 0.01$) and in liver ($P < 0.05$). Furthermore, the activities of CBX (interscapular fat, $P < 0.05$) and ME (perirenal, $P < 0.01$, and interscapular fat, $P < 0.05$) were higher and that of G6PDH (liver, $P = 0.07$) tended to be higher in females than in males.

Blood Measurements. The effects of CLA treatment and sex on plasma metabolites and leptin are shown in Table 6. Conjugated linoleic acid supplement reduced

Table 2. Carcass characteristics and meat quality of longissimus lumborum muscle 24 h postmortem from rabbits fed diets containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA) and slaughtered at age 76 d (2.5 kg BW)

	% Dietary CLA added			Sex		
Item	0	0.25	0.5	Male	Female	SEM
BW, g	2,525.8	2,526.2	2,522.5	2,537.5	2,512.2	45.8
CCW, g[a]	1,503.5	1,487.4	1,493.0	1,523.7	1,466.1	15.31
Dressing, %	59.5	58.9	59.2	60.0[c]	58.3[d]	0.45
Fat, g[b]						
Interscapular	7.9	8.3	8.9	8.3	8.5	1.68
Perirenal	18.9	17.4	19.0	18.9	18.0	1.39
pH	5.789	5.78	5.76	5.71	5.71	0.66
L*	56.28	55.65	54.23	54.66	56.11	0.66
a*	3.54	3.48	3.82	3.96	3.26	0.31
b*	2.84	3.01	3.91	2.72	3.12	0.27

[a]CCW = cold carcass weight 24 h after slaughter.
[b]Carcass weight was used as a covariate in the statistical analysis.
[c,d]Means in same row with different superscripts differ ($P < 0.01$).

($P < 0.05$) triglycerides and total cholesterol and tended to increase ($P = 0.06$) serum leptin concentrations. Higher levels of triglycerides were found in males than in females ($P < 0.01$), and a tendency ($P = 0.06$) for higher levels of leptin in females than in males was found.

Discussion

To improve meat quality, many producers in northern Italy slaughter rabbits at a higher body weight than in other countries. However, the heavier weight at slaughter adversely affects feed efficiency (which is very low during the finishing period) and also increases fatness, so that fat deposits, which are not appreciated by consumers, are evident on the carcass (Ouhayoun and Lebas, 1984). We tested CLA supplementation in rabbits to determine whether it could improve feed efficiency without adversely affecting carcass or meat quality. The trial was also designed to investigate the effects of CLA supplementation on carcass characteristics, particularly fat content, with slaughtering at three different times. We found that CLA supplementation did not have significant effects on average daily gain or on feed efficiency. However, the serial slaughtering design reduced the number of rabbits in the later stages of the study and may have reduced its power to reveal differences in growth. Our results are not consistent with the results of a number of studies in rats (Chin et al., 1994), mice (Park et al., 1997), or pigs (Cooper et al., 1998a; Ostrowska et al., 1999; Thiel-Eggert et al., 1999), all of which reported improved feed efficiency. Specifically, in those studies animals supplemented with CLA had reduced feed intake compared to controls but had similar (or higher) daily weight gain. The reasons for improved feed efficiency observed in these CLA-

Table 3. Carcass characteristics and meat quality of longissimus lumborum muscle 24 h postmortem from rabbits fed diet containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA) and slaughtered at 90 d of age (2.8 kg BW)

	% Dietary CLA added			Sex		
Item	0	0.25	0.5	Male	Female	SEM
BW, g	2,840.0	2,805.0	2,827.0	2,821.7	2,827.2	59.24
CCW, g[a]	1,712.8	1,703.7	1,716.8	1,702.2	1,719.1	21.76
Dressing, %	60.3	60.7	60.7	60.3	60.8	0.56
Fat, g[b]						
Interscapular	10.7	10.5	9.6	9.8	10.7	0.64
Perirenal[c]	26.7	22.7	21.5	22.8	24.8	1.67
pH	5.89	5.87	5.74	5.87	5.79	0.74
L*	63.0	61.9	63.6	61.7	64.0	1.24
a*	3.9	4.6	4.2	4.6	3.8	0.49
b*	3.1	2.9	3.6	3.1	3.3	0.35

[a]CCW = cold carcass weight 24 h after slaughter.
[b]Carcass weight was used as covariate in the statistical analysis.
[c]Dietary effect ($P = 0.089$).

Table 4. Carcass characteristics and meat quality of longissimus lumborum muscle 24 h postmortem from rabbits fed diet containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA) and slaughtered at age 104 d (3.2 kg BW)

	% Dietary CLA added			Sex		
Item	0	0.25	0.5	Male	Female	SEM
BW, g	3,243.6	3,182.6	3,203.5	3,243.3	3,181.00	85.29
CCW, g[a]	1,987.9	1,986.6	1,970.0	1,994.1	1,976.0	17.08
Dressing, %	61.4	62.3	61.5	61.4	62.1	0.60
Fat, g[b]						
Interscapular	11.9	12.6	11.4	11.7	12.5	0.91
Perirenal	33.8[c]	26.7[d]	24.9[d]	27.0[e]	30.1[f]	1.73
pH	5.96	5.91	5.97	5.95	5.93	0.60
L*	52.5	55.5	53.6	53.4	54.4	0.84
a*	1.80	1.94	1.10	1.69	1.52	0.31
b*	3.85	3.23	2.98	3.89	2.80	0.29

[a]CCW = cold carcass weight 24 h after slaughter.
[b]Carcass weight was used as covariate in the statistical analysis.
[c,d]Means in the same row with different superscripts differ ($P < 0.01$).
[e,f]Means in the same row with different superscripts differ ($P < 0.05$).

supplemented animals are not entirely clear. Some authors (Cook et al., 1993; Dugan et al., 1997) suggested this was due to the ability of CLA to regulate energy metabolism and nutrient partitioning. Cook et al. (1993) proposed that CLA supplementation protected against cytokine-induced muscle wasting by altering the eicosanoid (prostaglandin) pathway; and in fact it is now known that CLA administration lowers prostaglandin production in several tissues (Cunningham et al., 1997; Whigham et al., 2001).

In contrast with the studies cited above, Dugan et al. (1997) reported no significant influence of 2% CLA supplementation on growth performance in pigs. Thiel-Cooper et al. (1998a) found that average daily gain was not influenced in pigs fed diets with up to 0.5% CLA; whereas O'Quinn et al. (2000) found that in pigs fed

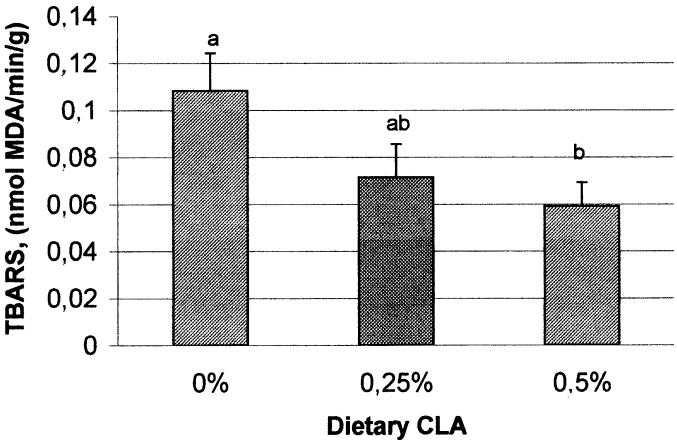

Figure 1. Effect of dietary conjugated linoleic acid (CLA) on thiobarbituric acid-reactive substances (TBARS) production following 140 min of Fe^{2+} catalyzed oxidation. The bars show mean ± SEM, n = 12. Bars not sharing a common superscript differ ($P < 0.05$).

0.50% CLA ADG was less, but not significantly, than in control animals in the live weight range of 37.6 kg to 72.6 kg. Greene et al. (2001) reported no effect of 0.5 or 2% CLA supplementation on daily gain, feed intake, or gain/feed in pigs.

With regard to carcass characteristics, the only effect of CLA supplementation in our study was that perirenal fat weight in rabbits slaughtered at medium and heavy live weights was lower than in controls. This finding is in agreement with previous observations showing reduced fat deposition in mice fed a CLA-supplemented diet (Park et al., 1997, 1999; Delany et al., 1999) or in pigs fed supplemental CLA (Dugan et al., 1997; Thiel-Cooper et al., 1998a, 2001). Conjugated linoleic acid may reduce body fat by several mechanisms, including reduced feed intake and increased metabolic rate. Reduced feed intake has been observed in CLA-fed mice and pigs, and this would partly explain the reduced fat content in carcasses. However, lower carcass fat in CLA-fed animals has been observed in other studies (in addition to the present one) characterized by unchanged energy intake (mice: Delany et al., 1999; pigs: Thiel-Cooper et al., 1998a).

An interesting finding of our study was that different regional fat deposits responded differently to supplemental CLA. Park et al. (1999) reported a similar effect in mice. Different body regions are known to differ in terms of adipose tissue metabolism and response to endocrine agents. Thus, in the rabbit, *de novo* lipogenesis is greater in mesenteric and perirenal adipose than in subcutaneous adipose tissue (Leung and Bauman, 1975; Gondret et al., 1997).

The total hepatic activity observed in our study was higher than that in the adipose sites, suggesting that liver is the major site of fatty acid synthesis; in fact, the relative contribution of liver to lipogenesis is about 70% in growing rabbits (Mourot, unpublished data). According to Vézinhet and Nouges (1977) and Leung

Table 5. Activity of acetyl-CoA-carboxylase (CBX), malic enzyme (ME), and glucose-6-phosphate-dehydrogenase (G6PDH) in liver and adipose tissues of rabbits fed a diet containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA) and slaughtered at 90 d of age

	% Dietary CLA added			Sex		
Item	0	0.25	0.5	Male	Female	SEM
CBX[a]						
Adipose tissue						
Perirenal	174.25[c]	73.69[d]	136.20[c]	111.97	140.66	14.74
Interscapular	92.36[c]	58.87[d]	49.90[d]	53.46[e]	80.61[f]	9.24
Liver	1,222.78[e]	1,402.86[e]	742.60[f]	987.99	1,287.79	145.19
ME[b]						
Adipose tissue						
Perirenal	19.23	24.21	22.24	16.99[c]	26.80[d]	2.48
Interscapular	12.76	17.06	14.14	11.58[e]	17.72[f]	2.16
Liver	288.025	305.15	383.69	361.49	334.50	55.70
G6PDH[b]						
Adipose tissue						
Perirenal	252.01	297.65	336.32	283.22	307.44	38.15
Interscapular	129.35	146.41	138.70	125.97	150.33	17.23
Liver	2,777.98	2,264.78	2,938.10	2,263.89	3,073.08	308.9

[a]Expressed as nmol HCO_3^- incorporated per minute per tissue.
[b]Expressed as μmol NADPH formed minute per tissue.
[c,d]Means in the same row with different superscripts differ ($P < 0.01$).
[e,f]Means in the same row with different superscripts differ ($P < 0.05$).
[g,h]Means in the same row with different superscripts tended to differ ($P = 0.07$).

and Bauman (1975), adipose tissue plays the major role in fatty acid synthesis only in rabbits older than 200 d of age.

The lower activity of CBX in liver and adipose tissues observed in our CLA-fed rabbits is also consistent with reduced perirenal fatness. Acetyl-CoA-carboxylase catalyzes the first step in fatty acid biosynthesis and seems to play a key role in the regulation of fatty acid biosynthesis by virtue of its being a rate-limiting enzyme for lipogenesis (Mourot et al., 1995). The effect observed on adipose tissue may also be related to a specific effect of CLA on adipose tissue and to an influence of CLA on plasma leptin, as discussed below.

The activities of CBX, ME, and G6PDH enzymes were lower in males than in females. This finding is consistent with the trend for female carcasses to have more fat than male carcasses.

Conjugated linoleic acid supplementation did not significantly affect the pH or color indices of LL muscle in our study. This is in agreement with previous observations showing no or minimal effects on meat quality in CLA-supplemented pigs (Carrol et al., 1999; Stahl et al., 1999) and a shelf-life study of loin chops from control and 1% CLA-supplemented pigs diets that found no differences in Hunter L*, a*, and b* values (Thiel-Cooper et al., 1998c). However, Dugan et al. (1999) reported slightly higher chroma values in pigs fed 2% CLA than in controls. Furthermore, Du et al. (2000) reported that chicken meat from birds supplemented with 1.25 to 5% CLA had better color after 7 d of storage. This might be due to improved oxidative stability of meat from CLA-fed chickens.

In our study CLA supplementation significantly increased the oxidative stability of LL muscle as evaluated by induced TBARS. In the backfat of piglets and in milk of sows fed CLA, Bee (2000a,b) found that saturated fatty acids were increased and unsaturated fatty acids reduced. A similar effect may have occurred in

Table 6. Plasma leptin and lipid profile in rabbits fed diet containing 0, 0.25, or 0.5% conjugated linoleic acid (CLA); animals were slaughtered at 90 d of age

	% Dietary CLA added			Sex		
Item	0	0.25	0.5	Male	Female	SEM
BW, g	2,840	2,805	2,827	2,822	2,827	59.2
Leptin, ng/mL	2.02	2.67	2.35	2.13	2.57	0.274
Total cholesterol, nmol/L	2.45[a]	1.87[b]	1.99[b]	1.94	2.26	0.242
Triglycerides, nmol/L	1.92[a]	1.40[b]	1.67[b]	1.97[c]	1.37[d]	0.181
NEFA, μmol/L	293.6	251.1	266.2	323.2[c]	217.4[d]	43.42

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).
[c,d]Means in the same row with different superscripts differ ($P < 0.01$).

rabbit muscle, which would account for the reduced oxidation we found. Du et al. (2000) also found that CLA treatment reduced lipid oxidation in raw chicken meat during storage. We found that total cholesterol and triglycerides were lower in CLA-fed rabbits than in controls. It is noteworthy that Lee et al. (1994) found less atherosclerosis in the aortas of CLA-fed than of control rabbits given a semisynthetic diet containing 14% fat and 0.1% cholesterol. The diet we used contained no added fat or cholesterol and we detected the anticholesterolemic effect after 5 wk of CLA treatment; Lee et al. (1994) observed reduced total and LDL cholesterol (and triglycerides) only after 12 wk.

We found that CLA treatment tended to increase plasma leptin ($P = 0.06$). This recently discovered protein hormone is produced and secreted by adipocytes (Klein et al., 1996; Ahima and Flier, 2000), and leptin receptors are found in many tissues. Leptin treatment has been shown to cause a dose-dependent decrease in food intake, loss of body weight, loss of fat depots, and increased energy metabolism (Pelleymounter et al., 1995; Levin et al., 1996).

Moya-Camarena et al. (1999) and Houseknecht et al. (1998a) reported that CLA activates the peroxisome proliferator-activated receptor-γ reporter gene (**PARR**γ), which partially controls transcription of the leptin gene repressing leptin synthesis (Houseknecht et al., 1998b). Furthermore, the pattern or the level of fatty acid in the diet also might affect leptin gene expression (Reseland et al., 2001), the leptin receptor function (Heshka et al., 2001), and the nuclear receptor PPARγ, which regulates transcription of leptin and several adipocyte-specific genes (Kallen and Lazar, 1996; Ryder et al., 2001).

In spite of the possible poor sensitivity of the leptin assay used in the present study, our findings on plasma leptin are consistent with the hypothesis that one mechanism by which CLA influences growth, fatness, and energy expenditure is via a change in leptin level.

Several studies had reported a relationship between CLA and plasma leptin in rats and mice. Raham et al. (2000) found that CLA reduced the high leptin levels in NIDDM (non-insulin-dependent-diabetes mellitus) rats. Delany et al. (1999) reported that 1% dietary CLA supplementation significantly reduced plasma leptin level in mice after 6 wk of dietary treatment, but not at 8 and 12 wk. Yamasaki et al. (2000) found that after only 1 wk of 2% dietary CLA supplementation, serum leptin levels were reduced in Sprague-Dawley rats fed a high-fat diet containing safflower oil. Safflower oil enhances the expression of leptin mRNA in rats and it was suggested that dietary CLA reduced this effect (Yamasaki et al., 2000).

Also, Tsuboyama-Kasaoka et al. (2000) observed in CLA-supplemented mice an increase of relative levels of PARRγ-mRNA in adipose tissue and a reduction of plasma leptin.

Dietary fat, level of CLA supplementation, different lengths of CLA supplementation, and species may all have contributed to the differences between our results in rabbits and those reported for other species. Clearly, further studies are required to elucidate the link between dietary CLA and serum leptin levels.

We found that circulating leptin levels tended to be lower ($P = 0.06$) but plasma triglycerides and NEFA were significantly higher in male than in female rabbits (slaughtered at 2.8 kg BW). The differences reflect sex hormone-related differences in lipid metabolism.

Finally, we noted that the CLA we used contained two major linoleic acid isomers: *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. It has been shown recently that different CLA isomers have different metabolic effects. In particular, reduced body fat in mice is associated with feeding *trans*-10, *cis*-12 CLA, and effects of CLA on lipolysis and lipoprotein lipase are apparently due to this isomer (Park et al., 1999).

Implications

Dietary conjugated linoleic acid may reduce carcass fat in rabbits weighing 2.8 kg or more. The oxidative stability of muscle tissue was also improved by conjugated linoleic acid supplementation, and this may favorably influence meat shelf-life. The alterations in lipid metabolism (modification of enzymatic activity and possible increase in leptin secretion) we found in this study suggest the need for further studies on the possible role of leptin as a mediator of the effects of conjugated linoleic acid on adipose tissue metabolism.

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