

## Conjugated Linoleic Acid in Combination with Supplemental Dietary Fat Alters Pork Fat Quality<sup>1,2</sup>

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**ABSTRACT** Interest in fortification of human foods, including pork, with conjugated linoleic acid (CLA) is growing and may provide benefits as a nutraceutical based on research evaluating CLA as an anticarcinogen, immune modulator, antiatherogenic agent and a body composition modulator. This study evaluated the combined effects of dietary CLA and supplemental fat source on growth, fatty acid composition and belly quality of lean genotype gilts ( $n = 144$ ). Pigs (49.3 kg) were randomly assigned to six diets ( $3 \times 2$  factorial) varying in supplemental fat (none, 4 g/100 g yellow grease or 4 g/100 g tallow) and linoleic acid [1 g/100 g corn oil (CO) or 1 g/100 g CLA (CLA-60)] for 47 d. Both the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 isomers of CLA were increased in belly and longissimus fat depots from pigs fed CLA, and that increase was up to 92% greater when CLA was fed with 4 g/100 g supplemental fat (fat source  $\times$  linoleic acid interaction,  $P < 0.05$ ). Pigs fed CLA had a greater concentration of 18:0 and less 18:1 *cis*-9 ( $P < 0.01$ ) in various fat depots, suggesting a reduction in  $\Delta^9$  desaturase activity. The iodine value of belly fat from pigs consuming tallow and CLA combined was reduced to 62.0 from an initial value of 70.4. CLA supplementation also increased belly weights ( $P < 0.05$ ). CLA did not affect longissimus muscle area, backfat depth and the percentage of fat-free lean ( $P > 0.10$ ), but it increased the subjective intramuscular fat score by 18.8% ( $P < 0.01$ ). In conclusion, CLA enrichment of pork products may be enhanced when combined with additional supplemental dietary fat, and together with tallow can be used to increase the saturated fatty acid content of pork. J. Nutr. 132: 3105–3112, 2002.

**KEY WORDS:** • conjugated linoleic acid • fatty acid composition • pork • supplemental fat • swine

The American Heart Association has recently recommended consumption of diets containing increased unsaturated to saturated fat ratios (U/S),<sup>4</sup> with up to 10% of energy intake as polyunsaturated fatty acids (PUFA) (1). Both classical studies and epidemiologic evidence were reviewed and it was concluded that (n-6) PUFA lower cholesterol and are protective against coronary heart disease and atherosclerosis (1). The swine industry has responded to these changing consumer preferences by developing leaner genotype pigs and by supplementing swine diets with unsaturated fat sources (2). However, increases in linoleic acid and other PUFA are undesirable to pork processors because increased PUFA content results in soft belly fat, which leads to poor bacon slicing and may result in increased rancidity. Therefore, we evaluated the effects of alternative supplemental dietary fat sources for

swine, including hydrogenated fat, tallow and conjugated linoleic acid (CLA) (3,4).

Development of pork products enriched with CLA may offset the negative effect of diets containing saturated fatty acids because several studies have shown that adding  $\leq 1$  g/100 g CLA to the diet can provide protection against cancer comparable with that provided by 18 g/100 g fish oil (5,6), whereas others have observed antiatherogenic effects (7). In addition, supplementation of swine diets with CLA has reduced backfat depth and improved belly firmness (8–12). Because CLA has the potential to alter gene expression of key lipogenic enzymes (13,14), including stearoyl-CoA desaturase (15), and supplemental fat can be directly deposited in swine adipose tissue, their combination may result in additive effects on pork fat composition and quality. Therefore, we tested the hypothesis that dietary CLA would increase the saturated/unsaturated ratio of pork fat and thus positively affect belly firmness. We further hypothesized that supplementation of a more saturated fat source [tallow; iodine value (IV) = 47] with CLA would further improve belly firmness beyond that occurring if CLA were combined with a more unsaturated fat source (yellow grease; IV = 83).

### MATERIALS AND METHODS

**Live animal care and measurements.** All animal procedures were approved by the Institutional Animal Care and Use Committee

<sup>1</sup> Presented in part at Midwest Animal Science Meetings, March 20, 2001, Des Moines, IA [Averette, L. A., See, M. T. & Odle, J. (2001) Conjugated linoleic acid supplementation increases belly weight in lean-genotype gilts. Proceedings p. 49.

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<sup>4</sup> Abbreviations used: CLA, conjugated linoleic acid; FFA, free fatty acid; IV, iodine value; LA, linoleic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; U/S, unsaturated to saturated fat ratio; wt, weight.

of North Carolina State University. Lean genotype gilts ( $n = 144$ ; mean weight, 49.3 kg) were delivered to the North Carolina Swine Evaluation Station from a large, integrated, North Carolina Pork Producer and were randomly blocked into one of two slaughter groups. Pigs were then randomly assigned to one of six treatments according to a  $3 \times 2$  factorial design (24 pigs per treatment; 3 pigs/pen). Diets (Table 1) were formulated to exceed NRC (16) nutrient requirements for finishing swine and included three sources of supplemental fat and two sources of linoleic acid (LA) as follows: 1) no supplemental fat + 1 g/100 g LA; 2) no supplemental fat + 1 g/100 g CLA; 3) 4 g/100 g yellow grease + 1 g/100 g LA; 4) 4 g/100 g yellow grease + 1 g/100 g CLA; 5) 4 g/100 g tallow + 1 g/100 g LA; and 6) 4 g/100 g tallow + 1 g/100 g CLA.

The source of CLA (kindly provided as a gift from Natural Lipids, Sandvika, Norway; www.natural.no) contained ~60% conjugated isomers as shown in Table 2. This product was chemically prepared from sunflower oil and was supplemented as a free fatty acid. Corn oil was chosen as the control because the LA content was also 60% (Table 2). The yellow grease and tallow had IV of 82.8 and 47.2, respectively. To ensure oxidative stability, the supplemented fats were stabilized with 0.1% ethoxyquin, and the interval between diet mixing and consumption by pigs was minimized ( $\leq 1$  mo). Dietary treatments (Table 1) were initiated after a 1-wk acclimation period. Pigs were divided into two groups by weight ( $n = 72$ ) with one group beginning treatment and being slaughtered 1 wk before the second group. Pigs consumed food and water ad libitum for 6 wk until they reached an average slaughter weight of 113 kg. They were weighed before initiation of treatments and then every 2 wk until slaughter. Feed consumption was recorded throughout the study. Backfat depth and longissimus muscle area were measured by real-time ultrasound (ALOKA 500V; Animal Ultrasound Services, Ithaca, NY) immediately before slaughter.

**Carcass measurements.** Hot carcass weight was determined at slaughter. Carcasses were chilled for 24 h at which time a 2.5-cm loin chop was removed between the 9th and 10th ribs. After allowing a minimum of 20 min bloom time, each chop was evaluated for color, ultimate pH and marbling score. The longissimus muscle chop was measured in triplicate (middle, medial and lateral) and mean values were calculated for color lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) using a Minolta Chromometer 200 (Minolta, Ramsey, NJ). The chromometer was set to D65 illuminant, a  $2^\circ$  standard observer, using

TABLE 1

Diet composition (as-fed basis)

Ingredient	Supplemental fat	Supplemental fat
	0 g/100 g	4 g/100 g
	<i>g/kg</i>	
Corn	808.7	733.9
Soybean meal, 48% crude protein	154.3	190.0
Corn oil or CLA-60	10.0	10.0
Tallow or yellow grease	—	40.0
Dicalcium phosphate	9.0	8.8
Limestone	8.4	8.3
NaCl	5.0	5.0
L-Lysine, 98%	1.6	1.0
V/VM premix <sup>1</sup>	2.5	2.5
Antibiotic (CTC)	0.5	0.5
Calculated composition		
Linoleic acid, g/kg	4.5	52.6
Metabolizable energy, kJ/kg	14,143	14,876
Lysine, g/kg	8.390	8.63

<sup>1</sup> Provided the following (mg/kg): 880 Cu as CuSO<sub>4</sub>; 4400 Fe as FeSO<sub>4</sub>; 2200 Mn as MnO; 4400 Zn as ZnSO<sub>4</sub>; 120 Se; 112 Iodine; 665 retinyl acetate; 11.1 cholecalciferol; 5909 vitamin E; 818 menadione; 15.5 biotin; 6600 choline; 136 folic acid; 880 niacin; 700 pantothenic acid; 450 pyridoxine; 176 riboflavin; 229.3 thiamine; 8.73 vitamin B-12.

CLA, conjugated linoleic acid.

TABLE 2

Fatty acid composition of supplemental fats

Fatty acid	Corn oil	CLA-60	Yellow grease	Tallow
			<i>g/100 g</i>	
8:0	0.13	0.16	0.14	0.13
10:0	ND	0.12	ND	0.14
12:0	ND	0.60	0.15	0.10
14:0	ND	0.24	1.68	4.98
14:1	0.07	0.07	0.35	1.24
16:0	10.25	4.57	20.16	25.90
16:1 <i>cis</i> -9	0.07	ND	5.12	4.66
18:0	1.40	1.77	6.20	14.08
18:1 <i>trans</i> -9	ND	ND	5.60	2.96
18:1 <i>cis</i> -9	25.38	18.36	33.63	34.58
18:2 <i>cis</i> -9, <i>cis</i> -12	60.29	6.86	21.12	4.26
18:2 <i>cis</i> -9, <i>trans</i> -11	ND	23.44	ND	ND
18:2 <i>trans</i> -10, <i>cis</i> -12	ND	29.63	ND	ND
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	1.06	ND	1.54	0.40
20:0	0.13	ND	ND	ND
20:1 <i>cis</i> -9	0.07	0.16	0.15	0.08
22:0	ND	ND	0.15	ND
SFA, <sup>1</sup> g/100 g	11.91	7.46	28.33	45.33
UFA, <sup>2</sup> g/100 g	86.94	78.52	67.51	48.18
U/SFA ratio	7.30	10.53	2.38	1.06
Iodine value <sup>3</sup> (IV)	134.35	125.24	82.78	47.18

<sup>1</sup> Saturated fatty acids: 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, and 20:0.

<sup>2</sup> Unsaturated fatty acids: 14:1, 16:1, 18:1, 18:2, 18:3, and 20:1.

<sup>3</sup> Calculated using the following equation (20): IV = 16:1 (0.95) + 18:1 (0.86) + 18:2 (1.732) + 18:3 (2.616) + 20:1 (0.785) + 22:1 (0.723).

CLA, conjugated linoleic acid; ND, not determined.

an 8-mm optical port with glass insert, and calibrated with Minolta white standard color plate. A visual color score was also determined on a scale from 1 to 6 (1 = pale, 6 = very dark) using plastic Japanese color standards (17). Japanese color standards are closely related to the Minolta  $L^*$  value but the scales are in the opposite direction. A lower Minolta  $L^*$  value indicates a darker color. Marbling scores were determined subjectively using a visual scale (1 = 1% intramuscular fat, 6 = 6%) (17). On the same sample, ultimate pH (24 h postslaughter) was measured using an Engold electrode and a K21 pH meter (NWK Binar, Landsberg, Germany).

The percentage of fat-free lean was estimated using a prediction equation for unribbed carcasses using the 10th rib loin muscle area and fat depth derived from real-time ultrasound and hot carcass weight (17). Drip loss (mg) was estimated by placing a preweighed Whatman #1 filter paper on a longissimus muscle section removed from between the 9th and 10th rib for 1 min. The filter paper was then reweighed and purge loss determined. The percentage of drip loss was estimated by the following equation: 48-h drip loss =  $-0.1 + (0.06) \times (\text{mg fluid}) \pm 0.09$ ;  $R^2 = 0.90$  (18). Initial backfat tissue cores were taken from each pig at the 10th rib, ~5 cm from the backbone and 2.5 cm deep as described previously (4). The initial content of fatty acids of the subcutaneous fat was as follows (g/100 g): 16:0 = 19.20; 16:1 = 5.84; 18:0 = 10.72; 18:1 *trans*-9 = 0.83; 18:1 *cis*-9 = 39.83; 18:2 *cis*-9, *cis*-12 = 17.25. Belly fat cores were removed from the shoulder end of the belly 24 h after slaughter ( $n = 8$ /treatment). Fat samples were placed in N<sub>2</sub> gas at the time of collection, stored at  $-80^\circ\text{C}$  and analysis was completed within 6 mo of collection. Lipids were extracted from longissimus muscle samples ( $n = 8$ /treatment) in duplicate before fatty acid analysis (19). Lipids were isolated from adipose tissue in duplicate and fatty acid composition was determined by gas-liquid chromatography as described previously (4). The IV was calculated from fatty acid composition data using the following equation (20): IV = 16:1 (0.95) + 18:1 (0.86) + 18:2 (1.732) + 18:3 (2.616) + 20:1 (0.785) + 22:1 (0.723). The IV represents the grams of iodine bound per 100 g of fat.

**Belly processing.** A subset of bellies ( $n = 48$ ; 8/treatment) were collected, squared and processed in a commercial facility. Bellies were weighed [fresh weight (wt)], pumped for a 20% increase in weight with a cure containing salt, sodium nitrite (6.25%), sodium erythorbate (2.5%), sugar, flavorings, FD & C Red #3 (0.00022%) and not > 1% sodium carbonate. After pumping, bellies were weighed (pumped wt) and then smoked for 24 h. Belly weights were recorded after smoking (smoked wt) to determine yield [(smoked belly wt/fresh belly wt)  $\times$  100].

**Statistical analysis.** Data were analyzed using the General Linear Models procedure of SAS (SAS Institute, Cary, NC). Least-squares treatment means were obtained assuming fixed models that included the effects of group, fat supplementation, LA source and fat supplementation  $\times$  LA source. The df were further partitioned into contrasts for the effects of supplemental fat source (yellow grease vs. tallow) and supplemental fat level (0 vs. 4 g/100 g). Differences were considered significant at  $P < 0.05$ .

## RESULTS

**Performance.** Feed consumption throughout the 47-d experiment was not affected by fat supplementation or addition of CLA to the diets ( $P > 0.10$ ) and averaged  $2.26 \pm 0.07$  kg/d. Average daily gain during the 47-d experiment was not affected by CLA consumption ( $0.87 \pm 0.03$  kg/d). Efficiency of gain (i.e., kg gain/kg feed intake) was improved by supplemental fat ( $0.37$  vs.  $0.40 \pm 0.01$ ;  $P < 0.01$ ). In addition, there was a fat source effect with a 2.6% improvement ( $P < 0.02$ ) in efficiency of gain in pigs consuming yellow grease compared with those consuming tallow.

**Carcass quality.** Backfat depth and longissimus muscle area were not affected by supplemental fat or CLA feeding (Table 3;  $P > 0.10$ ). Subjective marbling score was 18.8% greater in longissimus muscle chops from pigs fed CLA than in those fed LA ( $P < 0.01$ ). However, the estimated percentage of fat-free lean was not different between treatments ( $P > 0.10$ ).

**Fatty acid analysis.** CLA supplementation increased the weight percentages of 14:0, 16:0, 18:0 and 18:1 *trans*-9 and reduced the percentage of 18:1 *cis*-9 and 20:1 *cis*-11 ( $P < 0.001$ ) in belly fat samples (Table 4). The percentage of LA (*cis*-9, *cis*-2) was 12.5% greater in pigs consuming 4 g/100 g yellow grease ( $P < 0.001$ ) compared with pigs fed tallow. Both the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 isomers of CLA were increased in belly fat from pigs fed CLA, and that increase was even greater (ranging from 56 to 86%) when 4 g/100 g supplemental fat was fed (Fig. 1, fat source  $\times$  LA interaction,  $P < 0.001$ ). Total monounsaturates were reduced in belly fat

from pigs consuming CLA ( $P < 0.001$ ), whereas the total percentage of polyunsaturates was increased by 4 g/100 g supplemental fat ( $P < 0.05$ ), especially in those pigs consuming yellow grease ( $P < 0.01$ ). CLA supplementation reduced the ratio of monounsaturates to polyunsaturates [monounsaturated fatty acid (MUFA)/PUFA ratio] ( $2.50$  vs.  $2.30 \pm 0.05$ ;  $P < 0.001$ ). Addition of CLA to the diet increased the total amount of saturates, while reducing the U/S fatty acid ratio in the belly fat tissue ( $P < 0.001$ ). Overall, the IV also was affected by both fat and CLA; addition of CLA reduced belly fat IV by 6.6% ( $P < 0.001$ ) and the IV of fat from pigs consuming tallow and CLA combined was reduced to 62.0 (Fig. 2).

Similar to the belly fat analysis, pigs fed CLA also had reduced IV in samples from the longissimus muscle chops ( $P < 0.01$ ; Fig. 2). Supplementation of CLA increased the percentages of 14:0 and 18:0 ( $P < 0.01$ ) and decreased the percentages of 18:1 *cis*-9, 18:2 *cis*-9, *cis*-12 ( $P < 0.01$ ) and 18:3 *cis*-9, *cis*-12, *cis*-15 ( $P < 0.05$ ). Again, the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of CLA were increased by CLA feeding, especially in pigs fed 4 g/100 g supplemental fat (from 72 to 92%, Fig. 1; fat supplementation by LA interaction,  $P < 0.01$ ). Both the total monounsaturates and the MUFA/PUFA ratio were reduced by CLA supplementation ( $P < 0.01$ ). Feeding 4 g/100 g yellow grease increased total polyunsaturates in fat samples from longissimus muscle chops ( $P < 0.01$ ; Table 5). The total saturates increased and the U/S fatty acid ratio decreased in the intramuscular fat with CLA supplementation ( $P < 0.01$ ), comparable with the response in belly fat.

**Belly processing.** The green weight of the cut bellies, before cure injection, was increased 7.2% by CLA supplementation (Table 6;  $P < 0.02$ ). The increased belly weights from pigs fed CLA were maintained after pumping ( $P < 0.02$ ) and smoking ( $P < 0.08$ ). Supplemental fat source and level did not affect belly weights ( $P > 0.10$ ).

## DISCUSSION

Consumption of (n-6) PUFA has been associated with a reduced risk of coronary heart disease and atherosclerosis (1). Thus, consumption of diets containing increased (U/S) fat ratios has been recommended by the American Heart Association (1). Because pork fat can be modified through dietary fat alterations, the swine industry is searching for both effective and economical means of altering the fat composition of swine

TABLE 3

Carcass characteristics of pigs consuming 0 or 4 g/100 g supplemental fat combined with linoleic acid (LA) or conjugated linoleic acid (CLA)<sup>1</sup>

Item	Supplemental fat 0 g/100 g		Yellow grease 4 g/100 g		Tallow 4g 100 g		Pooled SEM
	LA	CLA	LA	CLA	LA	CLA	
Backfat, mm	13.89	13.57	15.29	15.03	14.34	13.80	0.79
Longissimus muscle area, cm <sup>2</sup>	40.05	39.60	41.60	41.22	42.31	39.54	1.10
Fat-free lean, <sup>2</sup> %	51.43	51.33	51.33	51.26	51.69	51.29	0.60
Marbling score <sup>3</sup>	1.61	1.85	1.73	2.34	1.77	1.88	0.16

<sup>1</sup> Values represent least-squares means and pooled SEM for  $n = 24$  pigs/treatment.

<sup>2</sup> Prediction equation for percentage fat-free lean (17):  $(1.006 \times (1 = \text{barrow or } 2 = \text{gilt}) - (18.838 \times \text{scan of 10th rib fat depth, in}) + (4.357 \times \text{scan 10th rib loin muscle area, in}) + (0.401 \times \text{hot carcass wt, lbs})$ .

<sup>3</sup> CLA > LA ( $P < 0.01$ ).

TABLE 4

Fatty acid profile of belly fat samples from pigs consuming 0 or 4 g/100 g supplemental fat combined with linoleic acid (LA) or conjugated linoleic acid (CLA)<sup>1</sup>

Item	Supplemental fat 0 g/100 g		Yellow grease (YG) 4 g/100 g		Tallow (T) 4 g/100 g		Pooled SEM	P-values			Contrast P-values g/100 g	
	LA	CLA	LA	CLA	LA	CLA		CLA	Fat	CLA × fat	0 vs. 4	YG vs. T
	g/100 g											
14:0	1.10	1.50	1.08	1.41	1.14	1.64	0.06	0.0001	0.0430	0.2715	0.6652	0.0139
16:0	20.37	22.81	20.03	20.52	19.43	21.72	0.43	0.0001	0.0097	0.0428	0.0031	0.0482
16:1 <i>cis</i> -9	1.86	1.94	1.82	1.87	1.78	1.91	0.10	0.2647	0.9045	0.9238	0.6588	0.9549
18:0	11.58	14.03	11.81	13.30	11.43	14.43	0.55	0.0001	0.7613	0.3861	0.8014	0.4901
18:1 <i>trans</i> -9	0.57	0.95	1.25	1.82	0.96	1.43	0.05	0.0001	0.0001	0.1087	0.0001	0.0001
18:1 <i>cis</i> -9	41.77	37.35	40.05	35.75	41.01	36.52	0.75	0.0001	0.0967	0.9912	0.0655	0.2490
18:2 <i>cis</i> -9, <i>cis</i> -12	16.02	14.88	17.08	17.56	16.04	14.72	0.48	0.0002	0.0002	0.1242	0.0337	0.0002
20:1 <i>cis</i> -11	0.77	0.65	0.70	0.59	0.72	0.63	0.03	0.0001	0.0604	0.7957	0.0291	0.3483
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.59	0.58	0.67	0.70	0.61	0.58	0.02	0.9143	0.0001	0.2813	0.0040	0.0001
Monounsaturates (MUFA)	45.0	40.9	43.9	40.1	44.5	40.5	0.80	0.0001	0.4300	0.9786	0.2788	0.4756
Polyunsaturates (PUFA)	16.7	16.4	18.1	20.0	17.0	17.1	0.50	0.1772	0.0001	0.0760	0.0012	0.0002
Saturates	33.43	38.67	33.20	35.50	32.27	38.08	0.80	0.0001	0.1215	0.0763	0.0721	0.3157
MUFA/PUFA ratio	2.7	2.5	2.4	2.0	2.6	2.4	0.10	0.0001	0.0002	0.4018	0.0022	0.0029
U/S ratio	1.86	1.49	1.90	1.70	1.91	1.52	0.06	0.0001	0.1211	0.2211	0.1251	0.1653

<sup>1</sup> Values represent least squares means and pooled SEM for  $n = 8$  pigs/treatment.

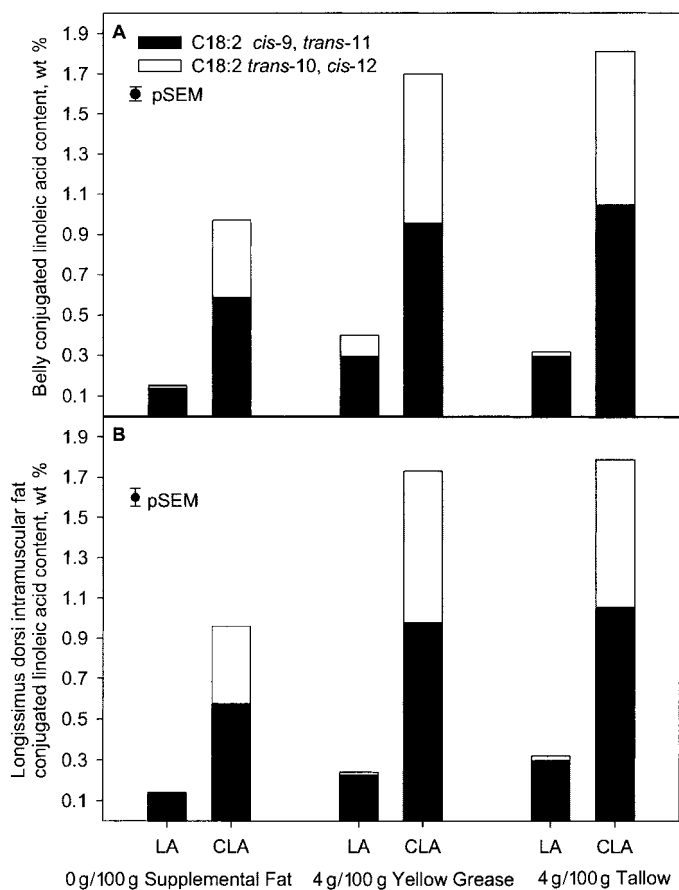
diets. The fatty acid profile of swine adipose tissue reflects that of the diet, and it is difficult to achieve an ideal (U/S) fatty acid ratio while accommodating the desires of both the consumer and pork processors (21). Interestingly, recent findings on the effects of consuming a relatively new PUFA, CLA, have shown that this fat has the potential both to affect human health and improve pork processing characteristics in a positive way (5–11). Therefore, there are two important issues that form the basis for the modification of pork fat by dietary means. First, CLA has immense potential as a nutraceutical; thus, improved understanding of ways to enrich the CLA content of natural human foods to create a functional food is warranted (22). Second, bacon consumption is increasing (23); thus, packers and processors are increasingly concerned about the quality of pork bellies stemming from soft fat depots (24). In this regard, supplementation of swine diets with CLA also has been shown to increase saturated fat content and thus pork belly firmness (8,9). Our study confirms the ease with which dietary fat alterations can be used to modify pork fat composition. In addition, data from our study show, importantly, that the CLA enrichment of pork could be enhanced when CLA was fed with additional supplemental dietary fat.

In response to consumer demands for pork products containing increased PUFA content, the swine industry has increased the number of genetically lean pigs to provide a lower fat product for consumers as well as more efficient production. Scott et al. (25) showed that genetically lean pigs have decreased endogenous fat synthesis. Thus, the relative proportion of fatty acids in adipose tissue of dietary origin increases in these pigs, making the characteristics of the fatty acids in the diet even more important. A decrease in endogenous fat synthesis partnered with an increased deposition of dietary fatty acids in pigs is beneficial for consumers who are concerned about health because these conditions will result in increased PUFA content in pork products. However, increasing the amount of PUFA in pork is at odds with the fat quality characteristics packers require to process high quality sliced

bacon and other processed products. Warnants et al. (21) have written an excellent review detailing the difficulty of manipulating the pork fatty acid profile with processing technology. Increased PUFA content also leads to a greater amount of lean and fat separation as well as smearing in grinding operations, which results in an undesirable product (26). For this reason, we chose lean-genotype pigs for this study. Indeed, in our study, the backfat depth was only 14 mm (96.9 kg carcass wt) on average, whereas the swine industry average is ~22.9 mm (83.5 kg carcass wt) (27).

In searching for solutions to improve fat quality, it is important to understand the main factors influencing the quality of backfat. Wood and Enser (28) showed that stearic acid was positively related to firmness and cohesiveness of fat, whereas LA was negatively related. This is probably because of an increase in membrane fluidity resulting from diets high in LA (29). Furthermore, lipogenic enzyme activities were increased in adipose tissue of pigs consuming diets with high levels of LA (30). Therefore, one possible solution to the soft belly fat problem is to supplement diets with a highly saturated fat source. Research in our laboratory has shown that feeding a chemically hydrogenated fat can reduce the PUFA composition of carcass fat and increase pork belly thickness as the saturation of supplemental fat increases (3).

Another solution yielding improvements in belly firmness has been the supplementation of diets with CLA (8,9). Because of the potential effects of CLA on human health, many researchers have recently evaluated CLA as a swine feed additive. CLA is a term used to describe a mixture of positional and geometric isomers of LA. Ruminant food products such as beef, milk and cheeses are natural sources of CLA. It is produced primarily by biohydrogenation by the ruminant bacteria *Butyrivibrio fibrisolvens* (31). However, synthetic sources of CLA are now available. Isomers of CLA have been reported to reduce tumor incidence, reduce body fat and increase body protein (11,12,32). Due to increasing interest of these and other potential effects of CLA on health, researchers have evaluated CLA supplementation in humans using cap-



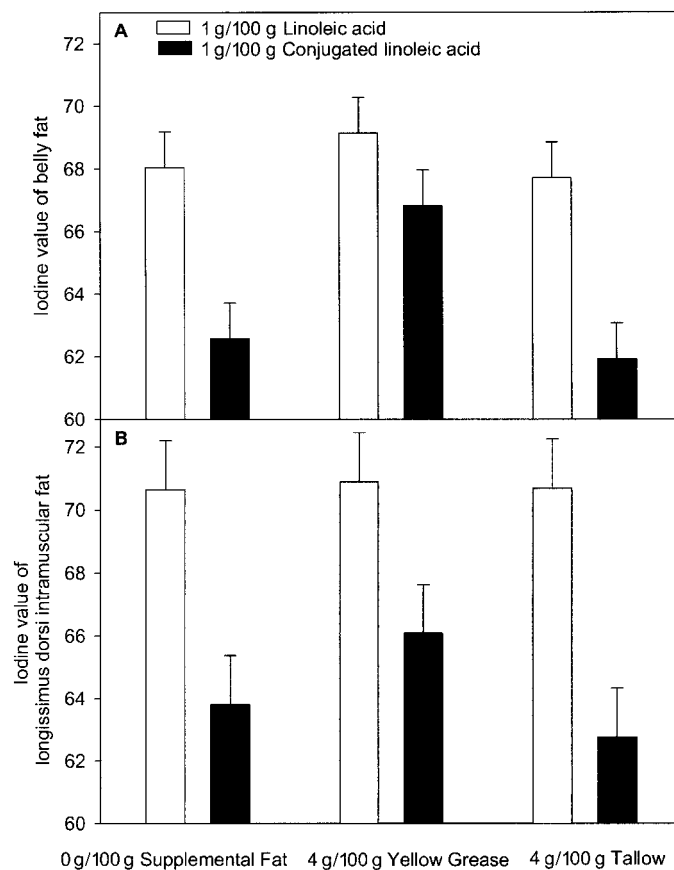
**FIGURE 1** Content of conjugated linoleic acid (CLA) isomers in belly fat tissue (A) and longissimus dorsi intramuscular fat (B) from pigs consuming 0 or 4 g/100 g supplemental fat combined with linoleic acid (LA) or CLA. Values are least-square means ( $n = 8$ ) of wt % (g/100 g fatty acid). The SEM for each isomer did not differ; therefore, the pooled SEM (pSEM) represents all means. Statistics for Panel A, both isomers: CLA > LA ( $P < 0.001$ ), fat level effect (0 vs. 4 g/100 g  $P < 0.05$ ), fat supplementation  $\times$  linoleic acid interaction ( $P < 0.05$ ). Statistics for Panel B, both isomers: CLA vs. LA ( $P < 0.001$ ), fat level effect (0 vs. 4 g/100 g;  $P < 0.01$ ), fat supplementation  $\times$  linoleic acid interaction ( $P < 0.01$ ); for 18:2 *cis-9, trans-11*, fat source effect (yellow grease vs. tallow;  $P < 0.01$ ).

sules containing < 1 g of CLA each (33). Although the current number of studies in humans is limited and the data are inconclusive, enrichment of foods with CLA might provide an opportunity for decreasing the risk of cancer and the incidence of obesity or to affect atherosclerosis, all of which have been demonstrated in animal research models.

If research continues to show beneficial effects of CLA consumption in humans and enrichment of CLA isomers in pork could be enhanced, a market for pork as a functional food may exist. We noted an increase in CLA isomers found in both belly fat and longissimus muscle lipid when CLA was supplemented as 1 g/100 g of the diet in this study. Others have also measured increases in both *cis-9, trans-11* and *trans-10, cis-12* LA isomers with CLA feeding (9,34). Interestingly, we measured even greater increases (ranging from 56 to 92%) in CLA content of belly fat and longissimus muscle lipid in pigs consuming 1 g/100 g CLA combined with 4 g/100 g supplemental fat compared with pigs consuming the diets containing 1 g/100 g CLA alone. This increase in CLA content of the tissue without a concomitant increase in CLA

intake has several possible explanations. The increase may have resulted from a reduction in CLA oxidation. Another possibility includes a lower digestibility of CLA, when supplemental fat is excluded, because the added CLA was fed as a free fatty acid (FFA) and FFA intake has been shown to affect digestibility in a negative manner (35). Alternatively, de novo fatty acid synthesis may have been reduced by supplemental fat feeding, allowing a greater deposition of dietary fatty acids (including CLA) compared with pigs consuming 1 g/100 g CLA without supplemental fat. Deposition of CLA in the latter group of pigs may be diluted by fatty acids synthesized de novo and stored in adipose tissue. Additional research is required to explain this important finding.

A linear relationship between the PUFA content of the feed and the PUFA content of both the backfat and intramuscular fat tissue appears to exist, although there are some differences in the incorporation rates of the two depots (4,21). In line with this finding, both supplemental fat and CLA addition to the diets resulted in changes to the fatty acid composition of belly fat and longissimus dorsi intramuscular fat. CLA supplementation increased 14:0, 16:0, 18:0 and 18:1 *trans-9* and decreased 18:1 *cis-9* and 20:1 *cis-11* in belly fat. In addition, the amounts of MUFA were reduced and the MUFA/PUFA ratio was increased by CLA feeding. Most other studies that have determined fatty acid composition after supplementing 1–2 g/100 g CLA have measured similar changes (9). In addition to measuring an increase in stearic



**FIGURE 2** Iodine value (g iodine bound/100 g fat) of belly fat (Panel A) and longissimus intramuscular fat (Panel B) from pigs consuming 0 or 4 g/100 g fat combined with linoleic acid (LA) or conjugated linoleic acid (CLA). Values are least-square means  $\pm$  pooled SEM (pSEM),  $n = 8$ . Statistics for Panel A: CLA vs. LA ( $P < 0.001$ ), yellow grease vs. tallow ( $P < 0.01$ ). Statistics for Panel B: CLA vs. LA ( $P < 0.01$ ).

TABLE 5

Fatty acid composition of longissimus dorsi intramuscular fat samples from pigs consuming 0 or 4 g/100 g supplemental fat combined with linoleic acid (LA) or conjugated linoleic acid (CLA)<sup>1</sup>

Item	Supplemental fat 0 g/100 g		Yellow grease (YG) 4 g/100 g		Tallow (T) 4 g/100 g		Pooled SEM	P-values			Contrast P-values	
	LA	CLA	LA	CLA	LA	CLA		CLA	Fat	CLA × fat	0 vs. 4%	YG vs. T
	g/100 g											
14:0	1.03	1.26	0.97	1.21	1.10	1.44	0.06	0.0001	0.0101	0.5300	0.5030	0.0032
16:0	19.70	21.53	20.15	19.30	19.24	20.99	0.74	0.1415	0.4890	0.1333	0.2847	0.5979
16:1 <i>cis</i> -9	1.73	1.81	1.74	1.65	1.82	1.78	0.08	0.7508	0.3978	0.5568	0.7505	0.1890
18:0	11.64	13.74	12.38	14.07	11.37	14.74	0.78	0.0006	0.7882	0.5423	0.5412	0.8307
18:1 <i>trans</i> -9	0.66	0.96	1.34	1.95	1.04	1.58	0.05	0.0001	0.0001	0.0112	0.0001	0.0001
18:1 <i>cis</i> -9	41.16	36.39	38.34	34.35	41.00	35.71	0.81	0.0001	0.0099	0.7230	0.0478	0.0170
18:2 <i>cis</i> -9, <i>cis</i> -12	17.73	16.06	18.83	17.87	17.60	15.63	0.53	0.0011	0.0053	0.6318	0.2142	0.0025
20:1 <i>cis</i> -11	0.78	0.67	0.73	0.63	0.74	0.59	0.03	0.0001	0.1361	0.7780	0.0547	0.6031
18:3 <i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15	0.65	0.62	0.73	0.70	0.66	0.58	0.03	0.0489	0.0012	0.5044	0.1593	0.0006
Monounsaturates (MUFA)	44.36	39.86	42.20	38.63	44.64	39.66	0.87	0.0001	0.0910	0.7198	0.2806	0.0552
Polyunsaturates (PUFA)	18.52	17.63	19.79	20.30	18.57	17.99	0.56	0.4899	0.0018	0.4383	0.0303	0.0033
Saturates	32.65	36.82	33.74	34.83	31.96	37.04	1.45	0.0044	0.9453	0.3177	0.8421	0.7889
MUFA/PUFA ratio	2.41	2.27	2.14	1.91	2.41	2.21	0.05	0.0001	0.0001	0.6841	0.0006	0.0001
U/S ratio	1.93	1.59	1.93	1.71	1.99	1.55	0.09	0.0001	0.7579	0.4649	0.6259	0.5760

<sup>1</sup> Values represent least-squares means and pooled SEM for  $n = 8$  pigs/treatment.

acid, we noted a corresponding decrease in oleic acid in fat samples from CLA-supplemented pigs. Ramsay et al. (34) showed similar results with apparent increases in the relative percentages of stearic acid and a reduction in oleic acid. They and others noted that 1–2 g/100 g dietary CLA may inhibit  $\Delta^9$  desaturase activity in both skeletal muscle and adipose tissue of several species (15,36). The  $\Delta^9$  desaturase enzyme catalyzes the synthesis of oleic acid (18:1 *cis*-9) from stearic acid (18:0); therefore, it is an important factor in determination of the levels of these fatty acids in porcine adipose tissue. This enzyme also has activity for synthesis of 14:1 *cis*-9 and 16:1 *cis*-9 from 14:0 and 16:0, respectively. CLA has been also been shown to reduce fat deposition and increase carnitine palmitoyl transferase enzyme activity in mice (13,37). Increases in the *cis*-9, *trans*-11 and the *trans*-10, *cis*-12 CLA isomers from belly fat of pigs in this study have been seen in other studies (8,9). When CLA concentration was increased linearly in diets from 0 to 1 g/100 g, a linear increase in the CLA content in both subcutaneous pork adipose tissue and lean tissue was found (8). Because CLA has the potential to alter gene ex-

pression of key lipogenic enzymes (13–15) and supplemental fat can be directly deposited in swine adipose tissue, their combination may result in additive effects on pork fat quality. Indeed, we noted that supplementation of 1 g/100 g CLA and 4 g/100 g tallow resulted in an additive reduction of belly fat iodine value (IV) (Figure 2A). Before this study, we and others have noted increased saturated fatty acid composition due to tallow supplementation (2,4,38). Pigs fed diets containing extruded full-fat soybeans or 4 g/100 g tallow for 6 wk exhibited a 39.9% reduction in LA of bacon samples from the pigs consuming the 4 g/100 g tallow diet compared with those consuming the 20 g/100 g full-fat soybean diet (2).

In our experiment, the 12.5% increase in LA from belly fat of pigs consuming 4 g/100 g yellow grease is not surprising. Boyd (39) compared the relationships between the fatty acid profile of the diet and the resulting profile and IV of backfat. He determined that there was a linear relationship between dietary LA content and the IV of backfat. These same relationships can be seen in this study. The yellow grease had a greater 18:2 *cis*-9, *cis*-12 content than the tallow, and tallow

TABLE 6

Belly weights during bacon processing of pigs consuming 0 or 4 g/100 g supplemental fat combined with linoleic acid (LA) or conjugated linoleic acid (CLA)<sup>1</sup>

Item	Supplemental fat 0 g/100 g		Yellow grease (YG) 4 g/100 g		Tallow (T) 4 g/100 g		Pooled SEM	P-values			Contrast P-values	
	LA	CLA	LA	CLA	LA	CLA		CLA	Fat	CLA × fat	0 vs. 4%	YG vs. T
Fresh wt, kg	2.44	2.65	2.57	2.68	2.59	2.81	0.09	0.0220	0.3084	0.8106	0.1800	0.4626
Pumped wt, kg	2.97	3.21	3.14	3.28	3.16	3.42	0.11	0.0241	0.2358	0.8713	0.1256	0.4684
Smoked wt, kg	2.20	2.41	2.36	2.42	2.40	2.58	0.10	0.0766	0.2345	0.7411	0.1560	0.3479
Yield, <sup>2</sup> %	90.0	90.5	91.7	90.2	92.4	91.9	1.2	0.6334	0.3192	0.6930	0.2609	0.3147

<sup>1</sup> Values represent least-squares means and pooled SEM for  $n = 8$  pigs/treatment.

<sup>2</sup> Yield = (smoked wt/green wt) × 100.

supplementation reduced the 18:2 *cis*-9, *cis*-12 content in both the belly fat and the longissimus muscle intramuscular fat. Other data collected in our laboratory have shown a linear decrease in 18:2 *cis*-9, *cis*-12 content and IV of carcass fat as dietary tallow level increased (4).

Both saturated fat and CLA have been shown to increase belly firmness (4,8,9) and have the potential to affect pork quality. The reduced IV and increased belly weights of pigs fed 1 g/100 g CLA in our study support an improvement in belly firmness. Eggert et al. (9) measured belly firmness on a scale from 1 (very soft) to 3 (very firm) in pigs fed 1 g/100 g CLA from 90 to 115 kg. Firmness was increased by 0.5 units in the CLA-fed pigs compared with those consuming diets with 1 g/100 g sunflower oil and by 0.8 units compared with those fed 1 g/100 g sunflower oil and restricted to the intake of CLA-fed pigs. Of the pork quality work completed, the main focus has been on the effect of CLA on longissimus thoracis quality and palatability (10,40). Several pork quality attributes, including marbling and intramuscular fat content, may be altered by CLA and dietary fat supplementation (40). The marbling score of the longissimus chops was increased 18.8% in pigs fed CLA (Table 3). Others have shown similar results with increases of 11.3% with CLA supplementation (41). Related to the increase in marbling score was a 2.77-g increase in intramuscular fat, partially resulting in an 18.0 g/kg wet loin increase in lean (42). Joo et al. (43) noted a reduction in purge loss of samples kept in cold storage for 7 d from pigs fed a 5 g/100 g CLA diet. This was related to an increase in intramuscular fat content and may be attributed to an increase in the resistance of membrane lipids to oxidation (43).

Several researchers have observed a reduction in backfat depth with CLA feeding (8,10,11,12). However, backfat depth in our study was not altered by CLA or supplemental fat and averaged only 14 mm at slaughter. Decreases in mRNA expression of fatty acid synthase, steroyl-coA desaturase and acetyl co-A carboxylase have been reported in mammary tissue of cows fed the *trans*-10, *cis*-12 CLA isomer, indicating that lipogenesis and fat deposition may be reduced by CLA (14). Backfat thickness in carcasses from pigs fed 4.8 or 9.5 g/kg CLA was reduced 24% compared with pigs receiving no CLA. However, backfat depths in these experiments were much greater (16–28 mm) than in our study. In addition, no effects of CLA were detected on the percentage of fat-free lean. Perhaps the pigs in our study were approaching a minimum backfat depth required for normal tissue structure and function and this may have resulted in increased resistance to further changes. Ramsay et al. (34) reported similar results in growing pigs consuming CLA and treated with porcine somatotropin. They did not detect an effect of CLA on body composition or on backfat depth. Along with our data, this would indicate that the greatest effect of CLA supplementation on body composition or total lipid content would be in pigs that are in the final stages of finishing or accumulating fat at a higher rate (34). Wood (44) posed two possibly relevant explanations, i.e., there may be a difference in the mechanism of fat deposition in genetically fat vs. genetically lean pigs or the proportion of de novo fatty acid synthesis (usually more saturated) is reduced in lean pigs along with an overall lower fat deposition at the same level of feed intake.

In summary, CLA enrichment of pork products may be enhanced when CLA feeding is combined with supplemental dietary fat in lean-genotype swine. In addition, data herein showed that, individually, tallow and CLA increased the saturation of belly fat and when supplemented together, reduced belly fat IV from 70 to 62 within 6 wk. Negative effects of tallow or CLA supplementation on growth, feed intake, feed

efficiency or carcass quality were not detected. Fatty acid composition of belly fat was altered by both tallow and CLA addition. However, supplementation of yellow grease increased the LA in belly fat samples and could increase the opportunity for lipid oxidation in pork products. Further research is warranted to investigate the metabolic basis for the extra CLA enrichment associated with dietary fat supplementation. Similarly, depot-specific effects (e.g., belly vs. backfat vs. intramuscular fat) merit further examination so that value-added enrichment of pork products may be optimized.

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