Journal of Animal and Feed Sciences, 20, 2011, 171–185

Effects of conjugated linoleic acid supplementation on growth, carcass characteristics and fatty acid profiles of muscle and fat in growing-finishing pigs*

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(Received 1 March 2011; revised version 6 April 2011; accepted 18 June 2011)

ABSTRACT

This study investigated the effects of dietary supplementation of conjugated linoleic acid (CLA) on the growth performance, carcass characteristics, muscle quality and fatty acid compositions in the skeletal muscle and backfat of growing-finishing pigs from 39.5 to 108.0 kg. Forty-eight pigs were randomly allotted to four diets containing 0 (control), 0.5, 1.0 or 1.5% of CLA mixture (35.2% *cis*-9, *trans*-11 and 37.5% *trans*-10, *cis*-12) for 12 weeks. The results showed that dietary CLA supplementation did not affect the animal's growth performance and carcass characteristics, whereas significantly increased dry matter content of the *Longissimus dorsi* muscle ($P<0.05$). The CLA supplementation also increased the content of saturated fatty acids, but decreased ($P<0.05$) the content of unsaturated fatty acids (UFA) in both the backfat and *Longissimus dorsi* muscle. The CLA concentrations in the *Longissimus dorsi* muscle and backfat were increased linearly by the CLA supplementation in a dose-dependent manner. However, CLA *cis*-9, *trans*-11 and CLA *trans*-10, *cis*-12 showed different deposition efficiencies in pigs.

* Supported by Hunan Science and Technology Major Projects, Grant No. 2009FJ1005, and the Knowledge Innovation Program (KIP) Important Projects of The Chinese Academy of Science, Grants No. KSCX-YW-N-49 and KZCX2-YW-T07
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KEY WORDS: conjugated linoleic acid, carcass characteristics, fatty acids, pigs

INTRODUCTION

Conjugated linoleic acid (CLA) refers to a group of positional and geometric (*cis* or *trans*) isomers of linoleic acid. It has been initially isolated and identified as an anticarcinogenic agent from fried ground beef (Ha et al., 1987). In recent years, CLA has been reported to have a wide range of biologically beneficial effects, including anticarcinogenic, antiobesity, antiatherogenic and immunomodulatory functions, and lean body mass promotion (Azain, 2003; Pariza, 2004; Bhattacharya et al., 2006).

Food products derived from ruminants are the richest natural sources of CLA (Tanaka, 2005). Researchers have demonstrated that dietary alterations of both ruminant and non-ruminant animals could increase the unsaturated fatty acid and CLA levels of meat, thereby improve its potential health benefits (Boles et al., 2005). The only plausible way to increase muscle CLA content effectively would be to provide chemically synthesized CLA as a dietary supplement in pigs (Joo et al., 2002). Thus, feeding pigs with CLA has been suggested as a potential strategy for obtaining meat products enriched with CLA (Schmid et al., 2006).

An important issue is the effect of dietary CLA on growth traits and fatty acid profiles. However, the results on this issue are inconsistent. Some researchers have shown the improvement in growth rate and carcass traits of pigs when fed diets enriched in CLA (Thiel-Cooper et al., 2001; Weber et al., 2006), but other studies did not find any significant responses (Ramsay et al., 2001; Tischendorf et al., 2002; Corino et al., 2003). On the other hand, few studies on the effects of dietary CLA on growth performance and fatty acids compositions have been conducted in pigs throughout grow-finish phase.

Thus, the present study was therefore designed to evaluate the influence of dietary levels of CLA supplementation on growth rate, carcass characteristics and fatty acid profiles in skeletal muscle and subcutaneous fat, and to determinate the optimum dietary CLA dose for growing-finishing pigs.

MATERIAL AND METHODS

Animals and diets

The use of the animals and the experimental procedure was approved by the Animal Welfare Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences.

Forty-eight Landrace × Large White pigs with the initial weight of 39.5 ± 1.7 kg were used for the study and randomly assigned to four dietary treatments in 12 animals with each. The animals were housed in individual pens, four piglets per pen (two barrows and two gilts in each pen), so each treatment had triplicates. Fresh water was accessed *ad libitum* (nipple drinkers).

The basal diet was composed to meet the nutrient requirements according the NRC (1998) standard and was purchased from a commercial feed company (Liuhe Ltd., Qingdao, China). Chemical composition and nutrient contents of the basal diet are shown in Table 1. The four diets were prepared by the basal diet supplemented with four levels of CLA (0.0, 0.5, 1.0 and 1.5%) (Institute of Geographic Sciences and Natural Resources Research, CAS, China). The CLA compound contained 72.7% CLA isomers (35.2% of *cis*-9, *trans*-11 and 37.5% of *trans*-10, *cis*-12 isomers).

Table 1. Ingredients and chemical composition of the basal diet

Item	%
Ingredients	
maize	65.2
soyabean meal	21.0
wheat bran	11.0
powdered limestone	0.8
dicalcium phosphate	1.2
salt	0.4
vitamin and mineral premix	0.4
Chemical composition	
dry matter	86.0
crude protein	15.0
crude fat	3.1
crude fibre	8.0
ash	8.0
calcium	0.7
phosphorus	0.4
lysine	0.8

The animals were fed twice at 08.00 and 18.00. The whole experimental period lasted for 13 weeks, consisting of one week of adaptation and 12 weeks of feeding period. Individual liveweight of pigs was recorded at the end of the experiment and used to calculate daily weight gain (ADG) over the experimental period. Pigs were slaughtered when their average weight reached 108 kg.

Sample collection and chemical analysis

At the end of the experiment, two animals (one barrow and one gilt) from each

pen were randomly selected for slaughter, thus there were six animals slaughtered for each treatment. Pigs were electrically stunned and followed exsanguinations. The carcass was scalded, for easy removal of hair, and then eviscerated. Hot carcass weight was recorded. The dressing percentage was calculated as hot carcass weight as a percentage of the final liveweight.

The left loin starting at the third from last thoracic vertebra and extending back to 30 cm was removed from the carcass within 0.5 h post slaughter, and taken to the laboratory immediately. The *M. longissimus dorsi* sample was taken to measure chemical composition: dry matter, ash, crude protein and crude fat (ether extract); the analyses were performed according to AOAC (2000) methods.

Samples of subcutaneous backfat and *Longissimus dorsi* muscle of about 10 cm length ended at the third from last thoracic vertebra on the left side of the carcass were taken, frozen in liquid N_2, and stored at -80°C for fatty acid analysis. On the analysis the samples of the muscle (about 2 g) and backfat (about 1 g) were hemogenated. Then 10 ml of extraction solution (chloroform:methanol, 1:1, vol/vol) and 3 ml distilled water were added and the extraction was allowed for 2 h at room temperature. After vacuum filtration, the residue was rinsed with 10 ml of chloroform and then filtered again. The filtered solution was pooled and centrifuged at 5500 *g* for 10 min. The upper layer (methanol and water layer) was removed and the bottom layer (chloroform layer containing lipid extracts) was sucked into a test tube. The tube was placed in a water bath at 37°C for evaporating chloroform under nitrogen stream. Then the extract was stored at -40°C until analysis of fatty acids.

Fatty acids in the extract were analysed as fatty acid methyl esters (FAME) by gas chromatography. Briefly, 2 mg of the extract was placed in a glass vial, and then 1.5 ml hexane, 100 µl methyl acetate and 100 µl sodium methoxide were added in order, and thoroughly mixed. The mixture was kept for 20 min at 20°C, and then 10 min at -20°C in a refrigerator. Afterward, 60 µl of oxalic acid was added quickly, and the vial was vortexed. After centrifuging at 5500 *g* for 10 min the supernatant was added with Na_2SO_4 to absorb moisture and stored for gas chromatography analysis.

The FAMEs was analysed by using an Agilent 6890N gas chromatographer, equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). A CP-Sil 88 fused silica open tubular capillary column (100 m×0.25 nm) (Chrompack, Bridgewater, NJ, USA) was used. The oven temperature started at 45°C for 4 min, thereafter, it was raised to 175°C at 13°C /min, held at 175°C for 27 min and then increased to 215°C at 4°C/min and then held at 215°C for 35 min.

The injector and detector temperatures were set at 250°C. Carrier gas was hydrogen at a flow rate of 30 ml/min. Identification of individual FAMEs was

accomplished by the retention times of an authentic standard. Four specific isomers of CLA (*cis*-9, *trans*-11; *trans*-10, *cis*-12; *cis*-9, *cis*-11; and *trans*-9, *trans*-11; Matreya LLC, USA) were identified. The concentration of individual fatty acids was quantitated according the peak area, and expressed as proportion to total fatty acids detected.

Statistical analysis

Statistical analysis of experimental data was performed by using the GLM procedure of SAS (1999). Dietary treatment was treated as a factor. For growth rate of pigs, there were three replicates (i.e. three pens). As for carcass traits and fatty acids, there were six replicates which came from six pigs slaughtered. Significant level of the treatment was set $P<0.05$, and significance of differences between the treatment means was tested using Duncan's test. A contrast analysis was also done to compare the linear (L) or quadratic (Q) response to CLA inclusion levels.

RESULTS AND DISCUSSION

Animal's growth performance, carcass traits and chemical composition of *Longissimus dorsi* muscle are shown in Table 2. The average feed intake and ADG were not affected by the inclusion of CLA ($P>0.05$). Similarly, Ramsay et al. (2001), Tischendorf et al. (2002) and Weber et al. (2006) found average daily gain of pigs was not influenced by feeding CLA. On the contrary, Thiel-Cooper et al. (2001) reported a linear increase in average daily gain in response to the level of CLA in the diet.

Table 2. Effects of dietary CLA supplementation on average daily gain, carcass characteristics, chemical composition of *Longissimus dorsi* muscle

Item	Dietary treatments CLA, %				SEM	P	Contrasts	
	0	0.5	1.0	1.5			linear	quadratic
Initial liveweight, kg	39.6	39.4	39.7	39.4	1.228	0.997	0.941	0.944
Final liveweight, kg	104.6	103.7	97.8	101.7	4.461	0.712	0.471	0.594
Feed intake, kg/d	1.84	1.97	1.87	1.84	0.062	0.461	0.776	0.254
Average daily gain, kg/d	0.76	0.71	0.65	0.69	0.049	0.453	0.221	0.377
Hot carcass weight, kg	76.8	78.4	74.4	76.3	3.545	0.885	0.744	0.972
Dressing percentage	73.4	75.6	76.1	75.1	0.851	0.174	0.158	0.083
Chemical compositions of fresh meat, %								
dry matter	30.8[b]	36.1[a]	31.7[b]	36.5[a]	1.489	0.022	0.070	0.876
crude protein	27.9	30.8	27.4	31.2	1.459	0.177	0.327	0.782
ether extract	2.57	3.61	2.66	4.08	0.531	0.159	0.148	0.731
ash	1.25	1.45	1.27	1.40	0.080	0.2403	0.492	0.642

[a,b] means within a row with different superscripts are different ($P<0.05$)

The hot carcass weight and dressing percentage were not affected by dietary CLA level (P>0.05), which is in agreement with the results of Cordero et al. (2010). There were also no significant effects (P>0.05) of dietary CLA on the content of crude protein, ether extract and ash in the *Longissimus dorsi* muscle, but the dietary supplementation of CLA increased (P<0.05) dry matter content in *L. dorsi* muscle. Similar results are found by Migdal et al. (2004) where a significant increase in dry matter content in meat occurred in pigs fed 2% CLA.

In this study, there was a numerically higher intramuscular fat content in the *Longissimus dorsi* muscle due to dietary CLA supplementation. Higher intramuscular fat content in *L. dorsi* has also been observed with dietary CLA supplementation (Joo et al., 2002; Martin et al., 2008). However, Bee (2001), Weber et al. (2006) and Corino et al. (2008) reported that CLA supplementation to pigs did not affect intramuscular fat content in pork. Although no significant influence of dietary CLA supplementation on intramuscular fat content was observed, the higher intramuscular fat content appeared to contribute to higher dry matter content in the *L. dorsi* muscle of the CLA groups.

The effects of dietary CLA on growth performance of the animals, carcass and meat quality were not completely consistent with other studies. This might be due to the differences in the genetics, the isomeric composition of CLA, the concentration and duration of supplementation of CLA, or CLA × genetic population interaction (Mersmann, 2002; Corino et al., 2003).

The effect of dietary supplementation of CLA on fatty acid composition of *Longissimus dorsi* muscle is presented in Table 3. The increase of dietary CLA level increased (P<0.05) concentrations of C16:0, C16:1*cis*-9, C18:1*cis*-12, CLA*cis*-9, *trans*-11, and SFA, and SFA/UFA proportion, and decreased concentrations of C18:1*trans*-9, C18:3n-3, C20:1*cis*-11, C20:2n-6, and C20:4n-6, and UFA proportions. Moreover C16:0, C18:1*cis*-9, C18:1*cis*-11, SFA, UFA concentrations and SFA/UFA showed linear change with the increment of dietary CLA. Additionally, C18:2*cis*-9, *cis*-12 concentrations in CLA treatment groups were lower than in the control (P<0.05). Joo et al. (2002) mentioned that linoleic acid concentration in pork loin was significantly decreased by dietary CLA, possibly linoleic acid in pork loin was replaced by supplemented CLA. Besides, this might be due to CLA accelerating catabolism (β-oxidation, etc.) of C18:2*cis*-9, *cis*-12 or inhibiting the deposition of C18:2*cis*-9, *cis*-12 as its isomers. However, the concentrations of C14:0, C18:0, C20:0 and monounsaturated fatty acids (MUFA) proportions were not affected by the dietary supplementation of CLA.

The *Longissimus dorsi* muscle in the CLA groups had higher SFA contents, but lower UFA content, resulting in a higher SFA/UFA ratio (P<0.05) than that of the control. The increase of dietary CLA level led to a linear increase in CLA

Table 3. Effects of dietary CLA supplementation on fatty acid profiles in *Longissimus dorsi* muscle

Fatty acid, %	Dietary treatments				SEM	P	Contrasts	
	CLA, %						linear	quadratic
	0	0.5	1.0	1.5				
C14:0	2.04	2.16	2.31	2.60	0.259	0.478	0.132	0.756
C16:0	28.3^{b}	31.2^{ab}	31.9^{a}	32.5^{a}	1.018	0.039	0.008	0.274
C16:1*trans*-9	0.28	0.25	0.27	0.26	0.025	0.869	0.783	0.871
C16:1*cis*-9	3.78^{b}	4.96^{ab}	4.64^{ab}	5.78^{a}	0.522	0.089	0.025	0.972
C17:0	0.16	0.17	0.19	0.20	0.017	0.402	0.108	0.729
C17:1*cis*-10	0.11	0.11	0.11	0.14	0.017	0.481	0.290	0.268
C18:0	11.8	12.5	13.5	12.2	0.640	0.305	0.458	0.143
C18:1*trans*-9	0.51^{ab}	0.56^{a}	0.48^{b}	0.49^{b}	0.019	0.034	0.106	0.290
C18:1*trans*-11	0.33	0.39	0.42	0.39	0.036	0.337	0.173	0.230
C18:1*cis*-9	31.1	33.1	29.7	29.5	1.602	0.391	0.279	0.492
C18:1cis-11	3.81	4.03	3.44	3.85	0.262	0.466	0.706	0.733
C18:1*cis*-12	0.20^{b}	0.28^{a}	0.20^{b}	0.26^{a}	0.018	0.015	0.228	0.753
C18:2*cis*-9,*cis*-12	13.6	7.86	9.56	8.55	2.330	0.325	0.209	0.319
C20:0	0.16	0.16	0.14	0.16	0.015	0.581	0.579	0.627
C18:3*n*-3	0.26^{b}	0.35^{a}	0.25^{b}	0.23^{b}	0.022	0.007	0.098	0.021
C20:1*cis*-11	0.36^{a}	0.20^{c}	0.27^{bc}	0.32^{ab}	0.026	0.002	0.763	0.001
CLA*cis*-9,*trans*-11	0.25^{b}	0.33^{ab}	0.5^{ab}	0.57^{a}	0.093	0.093	0.015	0.958
CLA*cis*-11,*trans*-13	0.08	0.10	0.13	0.16	0.037	0.492	0.133	0.854
CLA*trans*-10,*cis*-12	0.06	0.09	0.18	0.18	0.040	0.106	0.020	0.805
CLA*cc*	nd	0.03	nd	0.01	-	-	-	-
C20:2*n*-6	0.24^{a}	0.16^{b}	0.16^{ab}	0.18^{ab}	0.024	0.115	0.131	0.072
C20:3*n*-6	0.24	0.10	0.14	0.15	0.042	0.182	0.227	0.111
C20:4*n*-6	2.32^{a}	0.94^{b}	1.45^{ab}	1.28^{ab}	0.434	0.172	0.196	0.174
Total CLA	0.40	0.55	0.80	0.91	0.163	0.136	0.023	0.890
$R_{trans\text{-}10,cis\text{-}12/cis\text{-}9,trans\text{-}11}$	0.11^{b}	0.25^{ab}	0.30^{a}	0.28^{ab}	0.056	0.115	0.041	0.177
SFA	42.5^{b}	46.2^{ab}	48.0^{a}	47.7^{a}	1.656	0.103	0.029	0.234
MUFA	40.4	43.9	39.6	41.0	2.078	0.505	0.785	0.638
PUFA	17.1	9.95	12.4	11.3	2.786	0.323	0.246	0.290
UFA	57.5^{a}	53.8^{ab}	51.9^{b}	52.3^{b}	1.660	0.103	0.029	0.234
PUFA/SFA	0.43	0.22	0.27	0.24	0.082	0.276	0.178	0.268
SFA/UFA	0.75^{b}	0.86^{ab}	0.94^{a}	0.92^{ab}	0.055	0.094	0.026	0.233
Δ9 index	0.46	0.46	0.43	0.44	0.015	0.370	0.176	0.848

^{a,b,c} means within a row with different superscripts are different (P<0.05); nd - not detectable

$R_{trans\text{-}10,cis\text{-}12/cis\text{-}9,trans\text{-}11}$, ratios of the CLA*cis*-9, *trans*-11 to the CLA*trans*-10, *cis*-12

CLAcc, CLA*cis*-8, *cis*-10; *cis*-9, *cis*-11, or *cis*-11, *cis*-13

Total CLA - the sum of CLA; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids; UFA - unsaturated fatty acids

cis-9, *trans*-11 (P=0.015) and CLA *trans*-10, *cis*-12 (P=0.039). The total CLA content in the muscle showed a linear increase with increased dietary CLA level (P=0.023). The CLA concentration in the muscle of 1.5% CLA group (0.91%) was more than two times of the control group (0.40%).

The fatty acid profiles in the subcutaneous backfat are shown in Table 4. The dietary supplementation of CLA increased (P<0.05) the concentrations of C14:0, C16:0, CLA *cis*-9, *trans*-11, CLA *cis*-11, *trans*-13, CLA *trans*-10, *cis*-12,

Table 4. Effects of dietary CLA supplementation on fatty acid profiles in backfat

Fatty acid, %	Dietary treatments CLA, %				SEM	P	Contrasts	
	0	0.5	1.0	1.5			linear	quadratic
C14:0	2.30[b]	3[ab]	2.98[ab]	3.56[a]	0.2747	0.039	0.007	0.814
C16:0	27.8[b]	30.6[a]	30.9[a]	31.9[a]	0.926	0.033	0.008	0.354
C16:1*trans*-9	0.39	0.31	0.35	0.33	0.031	0.281	0.370	0.280
C16:1*cis*-9	2.10	3.22	2.02	2.11	0.410	0.145	0.538	0.229
C17:0	0.24	0.25	0.29	0.29	0.025	0.345	0.108	0.926
C17:1*cis*-10	0.16	0.14	0.13	0.13	0.018	0.672	0.279	0.646
C18:0	15.27	14.7	17.4	16.7	1.225	0.366	0.201	0.950
C18:1*trans*-9	0.6	0.56	0.46	0.74	0.094	0.258	0.459	0.105
C18:1*trans*-11	0.55	0.55	0.83	4.64	1.658	0.294	0.113	0.266
C18:1*cis*-9	32.3[a]	29.8[ab]	26.2[ab]	21.8[b]	3.191	0.158	0.027	0.774
C18:1cis-11	2.30[ab]	2.90[a]	1.97[ab]	1.66[b]	0.329	0.086	0.074	0.181
C18:1*cis*-12	0.14	0.17	0.17	0.15	0.021	0.568	0.647	0.199
C18:2*cis*-9,*cis*-12	12.7	10.9	12.0	11.1	1.077	0.604	0.442	0.660
C20:0	0.21[a]	0.16[b]	0.19[ab]	0.17[b]	0.012	0.046	0.109	0.167
C18:3*n*-3	0.40	0.47	0.51	0.50	0.061	0.584	0.228	0.569
C20:1*cis*-11	0.623	0.49	0.47	0.47	0.059	0.247	0.100	0.305
CLA*cis*-9,*trans*-11	0.65[b]	0.81[b]	1.44[ab]	1.76[a]	0.291	0.05	0.008	0.789
CLA*cis*-11,*trans*-13	0.12[b]	0.12[b]	0.28[ab]	0.33[a]	0.062	0.058	0.012	0.662
CLA*trans*-10,*cis*-12	0.26[b]	0.29[b]	0.71[ab]	0.88[a]	0.160	0.032	0.006	0.679
CLA*cc*	0.02[b]	0.04[b]	0.09[ab]	0.14[a]	0.029	0.043	0.006	0.574
C20:2*n*-6	0.47	0.42	0.43	0.43	0.050	0.877	0.563	0.654
C20:3*n*-6	0.10	0.08	0.10	0.08	0.013	0.610	0.331	0.824
C20:4*n*-6	0.30	0.16	0.16	0.12	0.080	0.408	0.151	0.520
Total CLA	1.06[b]	1.26[b]	2.53[ab]	3.11[a]	0.534	0.041	0.007	0.725
$R_{trans\text{-}10,cis\text{-}12/cis\text{-}9,trans\text{-}11}$	0.22	0.31	0.41	0.40	0.085	0.391	0.123	0.584
SFA	45.7[b]	48.6[ab]	51.7[ab]	52.6[a]	2.092	0.115	0.022	0.636
MUFA	39.1	38.1	32.6	32.1	2.870	0.218	0.055	0.934
PUFA	15.1	13.3	15.7	15.3	1.379	0.611	0.621	0.614
UFA	54.2[a]	51.4[ab]	48.3[ab]	47.4[b]	2.103	0.125	0.024	0.657
PUFA/SFA	0.33	0.27	0.30	0.29	0.029	0.482	0.456	0.405
SFA/UFA	0.86[b]	0.95[ab]	1.08[ab]	1.14[a]	0.079	0.098	0.016	0.836
Δ9 index	0.44[a]	0.42[ab]	0.37[ab]	0.31[b]	0.039	0.126	0.021	0.679

[a,b] means within a row with different superscripts are different (P<0.05) CLAcc, CLA*cis*8, *cis*-10; *cis*-9, *cis*-11, or *cis*-11, *cis*-13; total CLA - the sum of CLA; SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - poyunsaturated fatty acids; UFA - unsaturated fatty acids

CLAcc, total CLA, and SFA, and SFA/UFA proportion, and decreased the concentrations of C18:1*cis*-9, C18:1*cis*-11, C20:0 and UFA proportions, whereas the concentrations of C16:1 n-9, C18:2*cis*-9, *cis*-12 and C18:3n-3 proportions were not affected. Furthermore, there were linear relationships between the dietary CLA level and the C14:0, C16:0, C18:1*cis*-9, SFA, UFA concentrations and SFA/UFA.

The amounts of CLA *cis*-9, *trans*-11, CLA *cis*-11, *trans*-13, CLA *trans*-10, *cis*-12, CLAcc (*cis*8, *cis*-10; *cis*-9, *cis*-11, or *cis*-11, *cis*-13) and total CLA in the

backfat linearly increased ($P<0.05$) with the dietary CLA level. The concentration of total CLA in the backfat (3.11%) of 1.5% CLA group was more than two times ($P<0.05$) of the control group (1.06%).

The overall effects of dietary CLA supplementation on fatty acid composition in the backfat were similar to that in *Longissimus dorsi* muscle. The CLA supplementation resulted in greater SFA contents in the backfat, but lesser MUFA and UFA contents ($P<0.05$), therefore higher SFA/UFA ($P<0.05$) ratios than those of the control group. Adding CLA to pig diets increased belly fat firmness associated with an increased ratio of SFA:UFA (Eggert et al., 2001). Soft bellies can cause some difficulty during processing, decrease appearance and shelf-life of retail products (NPPC, 2000). The increase of SFA:UFA ratio could be of certain practical significance from a pork processing point of view (White et al., 2008).

Furthermore, there were some differences in the responses of MUFA and polyunsaturated fatty acids (PUFA) between the backfat and the muscle to CLA supplementation, where MUFA exhibited a decreasing tendency ($P=0.055$) in the backfat with the increment of dietary CLA, and this phenomenon was not observed in the muscle. Meanwhile, we observed that PUFA content in the *Longissimus dorsi* muscle of the CLA groups was lower than that of the control group. Interestingly, unlike the *L. dorsi* muscle, the 1 and 1.5% CLA supplementation had numerically higher PUFA content in the backfat compared with the control group. Therefore the decrease of UFA in the backfat and muscle might be mainly attributed to the changes in MUFA and PUFA. Above results also showed that the effects of dietary CLA supplementation on MUFA and PUFA contents differed between the fat and the muscle. It might be ascribed to the differences of lipid metabolism between the two tissues. Further research is needed to explain the mechanism on an increase of PUFA content in backfat of pigs fed CLA.

Previous experiments observed that an inclusion of CLA in the diet increased CLA *cis*-9, *trans*-11, CLA *trans*-10, *cis*-12, and SFA contents and decreased MUFA and PUFA in both backfat and muscle of pigs (Martin et al., 2007; Cordero et al., 2010). In this experiment it was not observed that the decreases of MUFA proportion in the muscle and PUFA proportion in the backfat with CLA supplementation. This is not in agreement with the previous study by Martin et al. (2007).

The effect of the decrease in UFA, especially MUFA, might be related to the inhibition of Δ9 desaturase activity and mRNA expression by CLA. The Δ9 desaturase index has been used as an estimator of Δ9 desaturase enzyme activity (Korniluk et al., 2007). In this study, the Δ9 desaturase index, defined as ratio of (C16:1*cis*-9 + C18:1*cis*-9) to (C16:1*cis*-9 + C18:1*cis*-9 + C16:0 + C18:0), numerically decreased with dietary CLA supplementation in both the *Longissimus dorsi* muscle and backfat, and the Δ9 desaturase index for the backfat decreased linearly with the dietary CLA levels ($P = 0.021$). However, the linear relationships

differed between the backfat and the muscle, with regression coefficients of -0.0892 and -0.0187 (Figure 1), respectively, for the backfat and the muscle, illustrating a reason for different changes in MUFA between the two tissues.

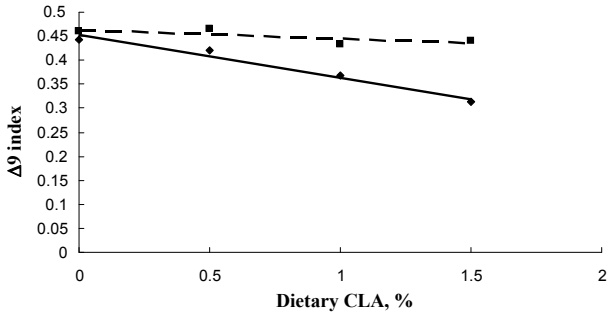

Figure 1. Linear response of Δ9 index in subcutaneous backfat (continuous line) and *Longissimus dorsi* muscle (doted line) to dietary CLA level. X = Dietary CLA%. Subcutaneous backfat: C18:1n-9/C18:0 = -0.0892X + 0.4529, R^2 = 0.9706; *Longissimus dorsi* muscle: C18:1n-9/C18:0 = -0.0187X + 0.4637, R^2=0.5936; Δ9 index: Δ9-desaturase index = (C16:1*cis*-9 + C18:1*cis*-9)/ (C16:1*cis*-9 + C18:1*cis*-9 + C16:0 + C18:0)

For the modified fatty acid profiles in adipose and muscular tissues, the direct substitution effect of dietary source on fatty acids in the body is one likely reason. Moreover the modulation of gene expression and activity of lipogenic enzymes and Δ9 desaturase by CLA supplementation are likely another explanation. It has been demonstrated that CLA reduced acetyl-CoA carboxylase activity in the liver and adipose tissues of rabbits (Corino et al., 2002). Corino et al. (2003) also reported that CLA supplementation significantly reduced the acetyl-CoA carboxylase activity in adipose tissue of pigs. Several studies have shown that dietary CLA supplementation inhibited the expression and activity of hepatic Δ9 desaturase (Lee et al., 1998; Choi et al., 2000; Shang et al., 2005). These results suggest that CLA has an inhibitory effect on Δ9 desaturase activity. Therefore, the reduction in MUFA content was a reflection of decreased C18:1 content and increased abundance of saturated fatty acids in the tissues of pigs fed CLA, and might be a direct result of the decrease in the desaturase activity (Corino et al., 2003).

The amounts of CLA *cis*-9, *trans*-11, *cis*-11, *trans*-13, and *trans*-10, *cis*-12 in the *Longissimus dorsi* muscle and backfat showed linear increases with dietary CLA levels. Even so, the mean concentration of CLA in the backfat (2.30%) was about three times of that in the *L. dorsi* muscle (0.75%) for the CLA groups.

Moreover, though CLA-isomers, *cis*-9, *trans*-11 and *trans*-10, *cis*-12 had similar contents in the diet, and CLA *cis*-9, *trans*-11 was slightly lower than

CLA *trans*-10, *cis*-12, a preferential incorporation of CLA *cis*-9, *trans*-11 was observed in this study. This phenomenon was also found by Thiel-Cooper et al. (2001) and Lauridsen et al. (2005). As shown in Figure 2, the responses of CLA isomers differed between the subcutaneous backfat and the *Longissimus dorsi* muscle. The concentrations of CLA isomers were lower in the *Longissimus dorsi* muscle than in the backfat. And CLA *cis*-9, *trans*-11 concentration was higher than CLA *trans*-10, *cis*-12 isomer in both the tissues. Both the isomers showed linear responses to dietary CLA supplementation.

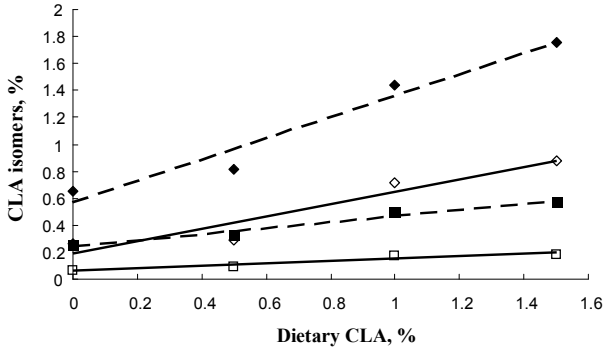

Figure 2. Linear response of CLA*cis*-9, *trans*-11 (doted line) and CLA*trans*-10, *cis*-12 (continuous line) concentrations to dietary CLA levels in subcutaneous backfat (◆, ◇) and *Longissimus dorsi* muscle (■, □), (◆), (◇), (■), (□) correspond to actual analysed values for subcutaneous backfat and *Longissimus dorsi* muscle, respectively. X - dietary CLA% ; subcutaneous backfat - CLA*cis*-9, *trans*-11 = (0.79 ± 0.25) X + (0.57 ± 0.23), P = 0.0054, R^2 = 0.955; CLA*trans*-10, *cis*-12 = (0.45 ± 0.14)X + (0.19 ± 0.13), P = 0.0043, R^2 = 0.9097
Longissimus dorsi muscle: CLA*cis*-9, *trans*-11 = (0.22 ± 0.08)X + (0.25 ± 0.07), P = 0.0108, R^2 = 0.9692 - CLA*trans*-10, *cis*-12 = (0.09±0.03)X + (0.06 ± 0.03), P = 0.0162, R^2 = 0.911

The ratios ($R_{trans\text{-}10,\ cis\text{-}12/cis\text{-}9,\ trans\text{-}11}$) of CLA *cis*-9, *trans*-11 to CLA *trans*-10, *cis*-12 in the subcutaneous backfat and *Longissimus dorsi* muscle of pigs fed the CLA diets were lower than the $R_{trans\text{-}10,\ cis\text{-}12/cis\text{-}9,trans\text{-}11}$ in the CLA compound that was added to the diets (i.e. 0.25-0.41 vs 1.07). Czauderna et al. (2004) also found the ratio of CLA *cis*-9, *trans*-11 to CLA *trans*-10, *cis*-12 in the liver of rats fed the diets enriched with CLA isomer mixture was lower compared with the ratio of these two isomers in the CLA isomer mixture (i.e. 0.712-0.833 vs 1.024). The accumulation rates of CLA *cis*-9, *trans*-11 and CLA *trans*-10, *cis*-12 differed between backfat and *Longissimus dorsi* muscle in piglets fed 1% CLA oil (Weber et al., 2006). As shown in Figures 3 and 4 there were linear

relationships between CLA *trans*-10, *cis*-12 and CLA *cis*-9, *trans*-11 content in the *L. dorsi* muscle and the subcutaneous backfat. The deposition rate for CLA *trans*-10, *cis*-12 was only 42.1% of that for CLA *cis*-9, *trans*-11 in the *L. dorsi* muscle (slope = 0.421), and 55.8% for the subcutaneous backfat (slope = 0.5577). The results of linear responses of CLA *trans*-10, *cis*-12 and CLA *cis*-9, *trans*-11 might have a value in CLA application. The CLA *trans*-10, *cis*-12 and CLA *cis*-9, *trans*-11, these two CLA isomers, have been shown to exhibit a wide range of physiological properties in cell culture and biomedical studies with a potential to improve long-term human health.

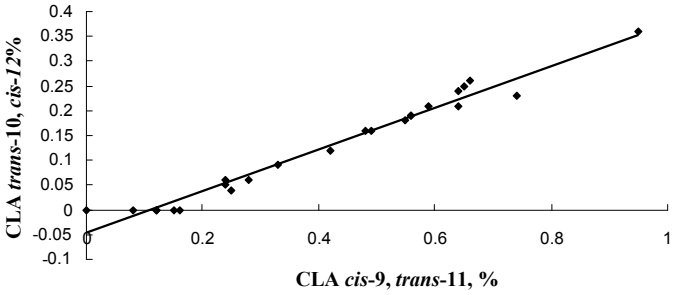

Figure 3. Linear relationship between CLA*cis*-9, *trans*-11 and CLA*trans*-10, *cis*-12 contents in *Longissimus dorsi* muscle. CLA*trans*-10,*cis*-12 = (0.4209 ± 0.01487)X + (-0.04612 ± 0.00712), P<0.0001, R^2 = 0.9733, where X - CLA*cis*-9, *trans*-11

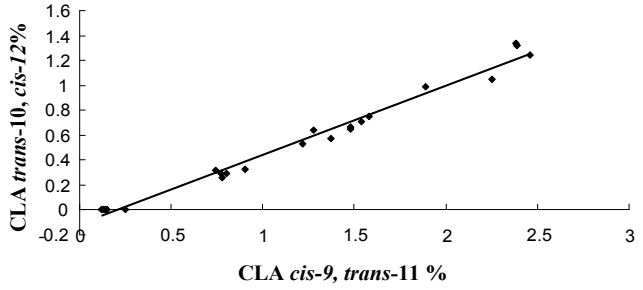

Figure 4. Linear relationship between CLA*cis*-9, *trans*-11 and CLA*trans*-10, *cis*-12 contents in subcutaneous backfat. CLA*trans*-10, *cis*-12 = (0.5577 ± 0.0158)X + (-0.1165 ± 0.0218), P<0.0001, R^2 = 0.9834, where X - CLA*cis*-9, *trans*-11

In the nutritional management practice by feeding CLA to pigs in order to improve CLA content in pork products, a high level of supplementation (about 1 time of CLA *cis*-9, *trans*-11) of CLA *trans*-10, *cis*-12 should be considered, if we wish to have the same enrichment of both CLA *trans*-10, *cis*-12 and CLA *cis*-9, *trans*-11 in porcine meat and fat.

The reason for the difference between CLA *cis*-9, *trans*-11 and *trans*-10, *cis*-12 content in tissues might be their dissimilar deposition efficiencies in pig. Czauderna et al. (2004) reckoned that CLA *trans*-10, *cis*-12 isomer is more efficiently driven through β-oxidation in cells of the muscles, kidneys, adipose tissue or liver of rats than their homologues. As for pigs, the varied deposition efficiencies of CLA-isomers are possibly attributed to their variations in absorption, transportation and catabolism (β-oxidation, etc.), or with competitive inhibition of CLA *cis*-9, *trans*-11 on CLA *trans*-10, *cis*-12 incorporation. Further study however, is needed to identify factors and precise molecular mechanisms of deposition and metabolism of CLA *cis*-9, *trans*-11 or CLA *trans*-10, *cis*-12.

CONCLUSIONS

The supplementation of diets with conjugated linoleic acid (CLA) did not notably affect growth performance, carcass characteristics, while it increased dry matter content in the *Longissimus dorsi* muscle in growing-finishing pigs. Furthermore, dietary CLA supplementation modified the fatty acid profiles in both the *Longissimus dorsi* muscle and backfat by increasing the concentrations of saturated fatty acids and decreasing the concentrations of unsaturated fatty acids, so the ratio of saturated fatty acids to unsaturated fatty acid increased. The CLA contents in the muscle and backfat increased in a CLA dose-dependent manner. The CLA cis-9, *trans*-11 and CLA *trans*-10, *cis*-12 displayed different deposition efficiencies in the muscle and backfat. Further studies should be undertaken to define the deposition mechanism of CLA isomers in pig tissues, and to determine the optimum CLA concentration in feed and time of feeding to achieve high CLA concentration in pig meat in a more economic way.

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