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Improvement in the carcass traits and meat quality of growing-finishing Rongchang pigs by conjugated linoleic acid through altered gene expression of muscle fiber types

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ABSTRACT. A total of 160 Rongchang pigs (26.76 ± 1.78 kg) were randomly assigned to 5 dietary treatment groups until their body weight (BW) reached 90 kg. The diets were supplemented with 0, 0.5, 1.0, 1.5, and 2.0% conjugated linoleic acid (CLA). Our results showed that the 1.0 to 2.0% CLA-fed pigs had less back fat deposition when their BW reached 90 kg than the pigs that received less than 1% CLA. During the 30 to 60 kg growing period, 1.0, 1.5, and 2.0% CLA treatments improved pork quality by significantly reducing the pork pH ($P < 0.01$) and color value ($P < 0.05$), but they increased marble scaling (P < 0.01). Similarly, the 1.5 and 2.0% CLA-fed pigs had more marble than other pigs when their BW reached 90 kg. Furthermore, CLA significantly affected the expression of muscle fiber-type genes. The 1.5% CLA-fed pigs exhibited the highest mRNA expression of MyHC1 and MyHC2a (P < 0.05) at 60 kg BW. At 90 kg BW, the highest expression of MyHC1 and MyHC2a (P < 0.05) was found in the 2.0% CLA group. However, MyHC2x was downregulated in the CLA-fed pigs at this time. In addition, CLA supplements did not evidently alter mRNA expression of MyHC2b at all times. These results demonstrate that CLA could affect carcass traits and improve the meat quality of growing-finishing pigs by altering the expression of genes related to muscle growth and development; 1-1.5% CLA was the most appropriate CLA dose.

Key words: Conjugated linoleic acid; Meat quality; Pig
Muscle fiber; Gene expression

INTRODUCTION

Conjugated linoleic acid (CLA), containing conjugated double bonds, comprises 18 carbon fatty acids that exist as a mixture of positional and geometric (*cis* or *trans*) isomers of linoleic acid (18:2 n-6). CLA has been extensively studied because of its beneficial effects on humans and animals, such as decreased body fat accumulation (Belury, 2002). Although as many as 56 possible isomers exist, only 2 (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) have received considerable attention because of their known biological effects (Hayashi et al., 2007).

Excellent meat quality is one of the most desirable traits of the Rongchang pig, which is indigenous to China. The intramuscular fat level in the Rongchang pig is almost thrice that of the European hybrid pigs (Lu et al., 2008). However, Rongchang pigs also have significantly higher back fat thickness than foreign breeds, which is unfavorable for pork production (Wang et al., 2011). Evidence has shown that CLA could reduce back fat thickness and increase intramuscular fat content in growing-finishing pigs (Thiel et al., 2001; Joo et al., 2002). In this study, we evaluated the overall effects of dietary CLA on growth performance, carcass traits, and pork quality as well as on the gene expression of growing-finishing Rongchang pigs. We also explored the optimal level of CLA in feeding.

MATERIAL AND METHODS

Animals and diets

A total of 160 Rongchang pigs (average body weight, 26.76 ± 1.78 kg) were randomly divided into 5 groups. Each group (32 pigs) was further randomly divided into 8 replicates of 4 pigs. Each group of pigs was fed a diet supplemented with 0, 0.5, 1.0, 1.5, and 2.0% CLA (source containing 61.2% free CLA, Qingdao Aohai Company, China). The profile of CLA isomers was 28.5 g c9, t11-CLA/100 g, 30 g t10, and c12-CLA/100 g. All diets were formulated to provide similar nutrition levels and according to the recommended nutritional needs for female pigs at certain ages (30 to 60 or 60 to 90 kg) by the Chinese meat-fat type pig feeding standard (NY/T 95-2004) (Table 1). One pig from each replicate was randomly selected for slaughter with final weights of 60 and 90 kg per replicate.

Table 1. Composition of the basal diets for pigs at different periods (air-dry basis, %).

Ingredients	30 to 60 kg	60 to 90 kg	Nutrient levels[b]	30 to 60 kg	60 to 90 kg
Corn	70.30	72.60	DE (MJ/kg)	13.84	13.83
Soybean meal	14.00	11.00	CP (%)[b]	15.95	13.95
Wheat bran	11.50	12.50	Ca (%)[b]	0.72	0.61
Soybean oil	2.00	2.00	TP (%)	0.54	0.47
Limestone	0.71	0.71	AP (%)	0.29	0.22
Calcium hydrogen phosphate	0.50	0.10	DLys (%)	0.71	0.49
NaCl	0.30	0.30	DMet (%)	0.21	0.19
Lysine	0.00	0.10	DMet+DCys (%)	0.43	0.39
Premix[a]	0.69	0.69	DThr (%)	0.43	0.37
Total	100.00	100.00	DTrp (%)	0.14	0.12
			DIle (%)	0.49	0.40

[a]Provided per kilogram of diet: Cu ($CuSO_4 \cdot 5H_2O$) 80 mg, Fe ($FeSO_4 \cdot 7H_2O$) 100 mg, Zn ($ZnSO_4 \cdot 7H_2O$) 100 mg, Mn ($MnSO_4 \cdot H_2O$) 40 mg, Se (Na_2SeO_3) 0.3 mg, I (KI) 0.3 mg, VA 1 750 IU, VD_3 200 IU, VE 11 IU, VK_3 0.5 mg, niacin 20 mg, pantothenic acid 9 mg, folic acid 0.3 mg, VB_1 1 mg, VB_2 3 mg, VB_6 1.5 mg, VB_{12} 15 μg, biotin 0.05 mg, choline chloride 1.0 g, phytase 0.1 mg, anti-oxidant 0.5 mg. [b]Measured value.

Carcass traits

Carcass traits and meat qualities were assessed in the left loin. The main detection indices include: carcass length, back fat depth between the 6th and 7th ribs, back fat depth at the 10th and last ribs, loin eye area, yield of carcass, leaf fat weight, and percentage lean content of the carcasses.

Meat qualities

The intramuscular fat (IMF) content of the longissimus dorsi muscle was determined using the Soxhlet extractor method. The shear force in the psoas and semitendinosus muscle was determined using a digital-display muscle tenderness determination device (C-LM3, Harbin, China). The water loss rate in the longissimus dorsi muscle between the 1st and 3rd lumbar vertebrae was measured using an unconfined pressure apparatus (WW-2A, Beijing, China). The longissimus dorsi muscle at the 6th and 10th thoracic vertebrae was removed for drip loss sampling.

The last 1-3 chest vertebrae of the longissimus muscle were selected for color, marble scaling, and pH value measurements. Tris-stimulus color coordinates, namely, lightness (L*), redness (a*), and yellowness (b*), were recorded using a Chroma Meter (CR-400, Minolta, Osaka, Japan). The marble was measured according to marbling standards (National Pork Producers Council). Then, pH1 at 30 to 45 min and pH2 24 h post-mortem were measured using SFK-Technology PH-STAR (DK-2730, Herlev, Denmark).

mRNA expression of regulators of muscle fiber type

The mRNA expression of some key factors in muscle fiber development (MyHC1, MyHC2a, MyHC2b, and MyHC2x) were measured using real-time polymerase chain reaction

(PCR), as described previously (Qi et al., 2012). Real-time PCR was performed using an ABI 7300 system (ABI, USA) with a 20-μL reaction mixture containing 10.0 μL SYBR Premix Ex Taq™ II (TaKaRa, Japan), 0.8 μL forward primer, 0.8 μL reverse primer, 2 μL template cDNA, 0.4 μL dye, and 6 μL ddH$_2$O. The cycling conditions comprised an initial single cycle of 30 s at 95°C, followed by 40 cycles of 5 s at 95°C and 35 s at the melting temperature (Table 2). To correct for the differences in the amounts of template DNA, the mRNA level is shown as a ratio to the β-actin mRNA level.

Table 2. Primer sequences of PCR.

Gene	Primer sequences (5'→3')	Tm (°C)
MyHC1	F: AAGGGCTTGAACGAGGAGTAGA R: TTATTCTGCTTCCTCCAAAGGG	58.2
MyHC2a	F: GCTGAGCGAGCTGAAATCC R: ACTGAGACACCAGAGCTTCT	57.0
MyHC2b	F: ATGAAGAGGAACCACATTA R: TTATTGCCTCAGTAGCTTG	55.6
MyHC2x	F: AGAAGATCAACTGAGTGAACT R: AGAGCTGAGAAACTAACGTG	55.0
β-actin	F: GCGGCATCCACGAAACTAC R: TGATCTCCTTCTGCATCCTGTC	56.3

Statistical analysis

Data were analyzed using the GLM procedure of SAS 9.0 (SAS Inst. Inc., Cary, NC, USA). Group differences were analyzed by least significant difference, and the differences were considered to be significant at P < 0.05.

RESULTS

Carcass traits

The carcass traits of pigs at 60 kg are shown in Table 3. Most of the traits exhibited no evident changes among groups, and only the 1.0 and 1.5% CLA-fed pigs exhibited a slight decrease in several traits, such as the vertical and incline lengths of the carcass and back fat depths at the shoulder-lumbar region between the 6th and 7th ribs as well as those at the 10th and last ribs. In addition, an increasing trend was observed on the loin eye area. Interestingly, the percentage lean content of the carcasses was remarkably increased in the 1.0, 1.5, and 2.0% CLA treatment groups (P < 0.01).

The carcass traits of pigs at 90 kg are shown in Table 4. We found that 1.0, 1.5, and 2.0% CLA significantly decreased back fat depth at the shoulder-lumbar region by 12.22 to 13.92% compared to that of the control. In addition, 1.0, 1.5, and 2.0% CLA also significantly decreased the back fat depth between the 6th and 7th ribs by 21.84% (P > 0.05), 7.76% (P > 0.05), and 15.80% (P < 0.01), respectively, compared to that of the control. Moreover, 1.0-2.0% CLA significantly decreased the skin-fat depth at the 10th rib by approximately 15.20 to 20.95% (P < 0.05). With increasing CLA level, a decreasing trend was observed on the fat-skin depth, but an increasing trend was found on the loin eye area. Similarly, 1.0 and 2.0% CLA significantly increased the percentage lean content of the carcasses compared with the control, and no significant difference was found among the other groups (P > 0.05).

Table 3. Effects of different conjugated linoleic acid (CLA) levels on the carcass parameters of pigs at 60 kg.

Items	Control	0.5% CLA	1% CLA	1.5% CLA	2% CLA	SE	P value
Body weight at slaughter (kg)	61.8	62.5	61.2	62.4	62.6	0.90	0.8151
Vertical length (cm)	78.5	80.1	80.0	81.2	78.8	1.13	0.4236
Incline length (cm)	67.9	68.5	69.7	69.4	67.1	1.13	0.5038
Back fat depth (cm)							
Shoulder-lumbar region	2.61	2.74	2.41	2.43	2.68	0.13	0.2800
Between the 6th and 7th ribs	2.66	2.54	2.41	2.42	2.62	0.16	0.7484
Fat-skin depth (cm)							
10th rib	2.68	2.65	2.51	2.30	2.48	0.17	0.5302
Last rib	1.99	2.00	1.80	1.86	1.95	0.16	0.8885
Loin eye area (cm^2)	18.40	18.61	19.73	20.55	20.23	0.86	0.3028
Yield of carcass (%)	69.3	70.6	70.2	69.5	70.8	0.54	0.1803
Leaf fat weight (kg)	1.52	1.70	1.52	1.49	1.56	0.10	0.6252
Percentage lean content of the carcasses (%)	48.0^b	47.9^b	51.5^a	50.2^a	51.5^a	0.65	0.0002

^{a,b}Different letters in same line mean significant difference (P < 0.05).

Table 4. Effects of different conjugated linoleic acid (CLA) levels on the carcass parameters of pigs at 90 kg.

Items	Control	0.5% CLA	1% CLA	1.5% CLA	2% CLA	SE	P value
Body weight at slaughter (kg)	90.9	90.3	89.6	89.9	89.8	1.17	0.9423
Vertical length (cm)	89.8	88.3	91.5	91.0	91.0	1.06	0.2124
Incline length (cm)	76.6	75.4	77.1	77.5	77.1	0.74	0.3064
Back fat depth (cm)							
Shoulder-lumbar region	3.52	3.35	3.05	3.09	3.03	0.15	0.0827
Between 6th and 7th ribs	3.48^a	3.49^a	2.72^c	3.21^{ab}	2.93^{bc}	0.14	0.0009
Fat-skin depth (cm)							
10th rib	3.58^a	3.58^a	2.83^b	3.00^b	2.83^b	0.17	0.0017
Last rib	2.75	2.78	2.31	2.40	2.38	0.16	0.1407
Loin eye area (cm^2)	22.46	22.45	24.19	24.92	24.91	0.87	0.1100
Yield of carcass (%)	74.2^a	73.3^{ab}	71.8^b	72.7^b	72.5^b	0.50	0.0217
Leaf fat weight (kg)	3.10^a	2.92^{ab}	2.52^b	2.60^b	2.48^b	0.14	0.0131
Percentage lean content of the carcasses (%)	47.9^b	50.1^{ab}	52.5^a	50.4^{ab}	50.8^a	0.87	0.0157

^{a,b,c}Different letters in same line mean significant difference (P < 0.05).

Meat qualities

The meat qualities of pigs at 60 kg are shown in Table 5. We observed that 1.0, 1.5, and 2.0% CLA significantly reduced the pH1 value (P < 0.01) by 4.53% (P < 0.05), 2.34% (P > 0.05), and 5.26% (P < 0.05), respectively, compared to that of the control. In addition, the 1.0% CLA-fed pigs had a lower b* value, and no significant difference was found among other treatments. Moreover, all CLA treatments significantly increased marble scaling by 10.82-32.47% compared with the control, and the 1.5% CLA-fed pigs exhibited the highest marble scaling (P < 0.05).

Table 5. Effects of different conjugated linoleic acid (CLA) levels on meat quality at 60 kg.

Items	Control	0.5% CLA	1% CLA	1.5% CLA	2% CLA	SE	P value
pH1	6.84^a	6.94^a	6.53^b	6.68^{ab}	6.48^b	0.10	0.0090
pH2	5.83	5.89	5.84	5.77	5.76	0.04	0.1596
Color	3.75	3.75	3.75	3.75	3.56	0.09	0.4817
L*	34.7	34.8	33.9	35.6	34.6	0.75	0.6095
a*	4.62	4.66	4.30	3.96	4.46	0.24	0.2364
b*	2.84^a	3.06^a	2.33^b	2.59^{ab}	2.74^{ab}	0.16	0.0335
Marbling	2.31^b	2.75^{ab}	2.56^{ab}	3.06^a	2.63^{ab}	0.17	0.0456
Intramuscular fat (%)	1.72^b	2.49^a	2.62^a	2.49^a	2.50^a	0.20	0.0229
Shear force (N)							
Semitendinosus	4.34	4.37	4.89	5.31	4.84	0.28	0.1117
Psoas major	2.12	2.58	2.40	2.60	2.75	0.21	0.3029

^{a,b}Different letters in same line mean significant difference (P < 0.05).

Table 6 shows the meat qualities of pigs at 90 kg. We found that 0.5-2.0% CLA significantly decreased the b* value of meat by 7.61-15.96% compared to that of the control, and the 1.0% CLA-fed pigs had the lowest b* value ($P < 0.05$). All CLA supplementations evidently increased marble scaling by 3.29-25.65% compared with the control, and the 1.5 and 2.0% CLA-fed pigs had higher marble scaling ($P < 0.01$).

Table 6. Effects of different conjugated linoleic acid (CLA) levels on meat quality at 90 kg.

Items	Control	0.5% CLA	1% CLA	1.5% CLA	2% CLA	SE	P value
pH1	6.29	6.38	6.34	6.42	6.35	0.09	0.8955
PH2	5.38	5.42	5.43	5.37	5.33	0.05	0.6505
Color	4.13	4.13	4.00	4.06	4.19	0.18	0.9575
L*	34.2	34.0	34.3	35.0	33.7	0.66	0.7291
a*	4.49	4.50	4.29	4.68	5.12	0.28	0.3264
b*	2.89^a	2.66^{ab}	2.40^b	2.67^{ab}	2.55^b	0.10	0.0223
Marbling	2.69^b	3.13^{ab}	2.94^{ab}	3.38^a	3.31^a	0.16	0.0239
Intramuscular fat (%)	2.19^b	3.21^a	2.93^a	3.10^a	3.08^a	0.22	0.0144
Water holding (%)	18.1	17.4	17.8	15.9	17.6	1.12	0.6647
Drip loss (%)	2.02	2.11	1.78	1.70	1.98	0.14	0.2135
Shear force (N)							
Semitendinosus	4.36	4.47	4.50	4.77	4.55	0.28	0.8713
Psoas major	3.58	3.10	2.90	3.11	3.15	0.21	0.2569

[a,b]Different letters in same line mean significant difference ($P < 0.05$).

Changes in mRNA expression of regulators in the muscle fiber development

Muscle fiber is an important structural unit of muscle, and differences in muscle fiber type significantly affect meat quality. As shown in Figure 1, compared with the control group of pigs at 60 kg, all CLA-fed pigs exhibited higher expression of MyHC1 and MyHC2a, and the 1.5% CLA-fed pigs has the highest mRNA expression level ($P < 0.05$). However, at 90 kg, the highest expression level of both MyHC1 and MyHC2a ($P < 0.05$) was found in the 2.0% CLA group (Figure 2). In addition, no significant changes in mRNA expression of MyHC2b and MyHC2x ($P > 0.05$) were observed at 60 kg, but the latter was downregulated in the 0.5% CLA-fed pigs at 90 kg.

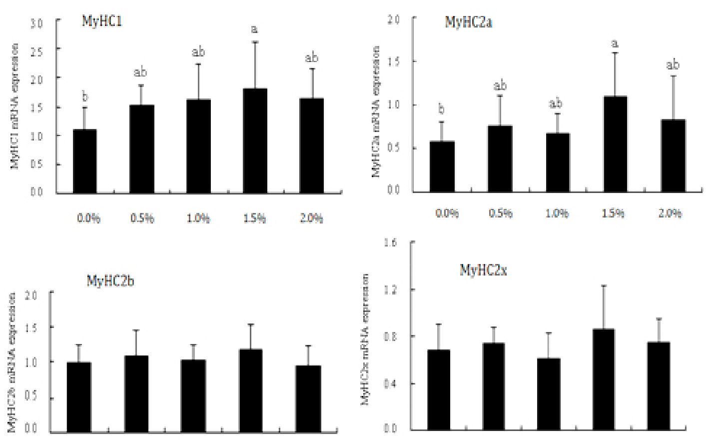

Figure 1. mRNA expression levels of regulators of muscle fiber development at 60 kg body weight. The mRNA levels were measured by real-time PCR analysis and normalized to β-actin. Values are reported as means ± SE; N = 5. Different letters on the bar mean $P < 0.05$.

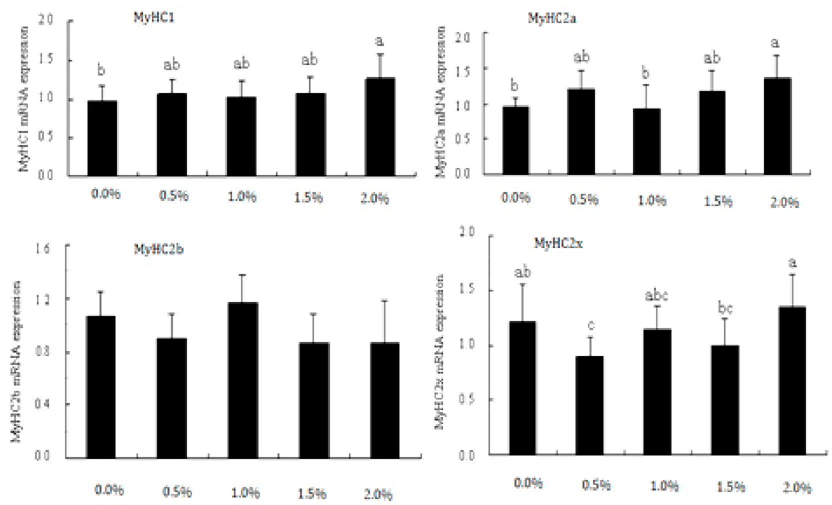

Figure 2. mRNA expression levels of regulators of muscle fiber development at 90 kg body weight. The mRNA levels were measured by real-time PCR analysis and normalized to β-actin. Values are reported as means ± SE; N = 5. Different letters on the bar mean $P < 0.05$.

DISCUSSION

Numerous studies have found that CLA significantly affects the growth, production, and health of animals (Decker, 1995; Pariza et al., 2000; De la Torre et al., 2005; Suksombat et al., 2006). Results from these studies suggested that CLA could inhibit the proliferation of tumor cells, reduce the occurrence of cardiovascular diseases, regulate the immune system and inflammatory responses, and decrease fat deposition via changes in the fat metabolism of animals. Therefore, CLA has been considered a beneficial nutritional additive for use in animal feeding.

In this study, we found that dietary CLA supplementation significantly affects the carcass traits of pig, especially fat deposition. Dietary CLA supplementation reduced fat deposition in some parts of the carcass, such as the back fat-depth in the shoulder-lumbar region. The percentage lean content of carcasses was higher in the CLA-fed pigs than in the control pigs, and the 1.0% CLA-fed pigs had the greatest value. An increasing body of evidence has proven that CLA serves a key function in the fat metabolism of animals (Azain et al., 2000; Miner et al., 2001; Hargrave-Barnes et al., 2008). Some experiments have shown that CLA decreased fat deposition by inhibiting adipocyte proliferation and differentiation (Ostrowska et al., 1999; Satory and Smith, 1999; Azain et al., 2000). In addition to fat deposition, CLA supplementation affects other indices of meat quality. In this study, the meat of CLA-fed pigs exhibited more IMF and marbling than control pigs. IMF content is an important determinant of meat quality and significantly affects other meat traits, such as tenderness, juiciness, flavor, and acceptability by consumers (Hovenier et al., 1993; Gao and Zhao, 2009; Barnes et al., 2012). Therefore, CLA supplementation to increase the percentage lean content of pig and increase the IMF of meat will improve meat quality and enhance the economic value of animal production.

We found that CLA supplementation changed the mRNA expression of some genes related to muscle fiber development and transformation. The growth and development of muscle fiber have important effects on meat quality. Several studies have found that the type and percentage of muscle fiber are importantly correlated with the color, pH, drip loss, and IMF content of meat. However, a few studies on the CLA regulation of muscle fiber growth and development did not show a clear correlation.

In previous studies, the muscle fiber of pigs was categorized into 4 types depending on the myosin type, namely, MyHC1, MyHC2a, MyHC2b, and MyHC2x (Bottinelli and Reggiani, 2000). MyHC1 is an oxidized-type muscle fiber, whereas MyHC2a and 2b are glycolytic-type muscle fibers. Different types of muscle fibers should transform into one another as the pig grows (Berchtold et al., 2000). In this study, the expression of MyHC1, MyHC2a, and MyHC2x was evidently changed by CLA supplementation. However, the changes differed at the same times. The percentage increase of MyHC2a muscle fiber was considered to be beneficial for meat quality. However, MyHC1 usually has higher expression in smaller pigs, and the reason why CLA supplementation upregulated MyHC1 expression remains unclear. However, we believe that appropriate CLA supplementation improves pig meat quality by indirectly affecting muscle fiber growth and development by altering gene expression.

In conclusion, dietary CLA supplementation decreased the back fat deposition, increased the lean content of the carcasses, and improved meat quality by modifying the mRNA expression of muscle fiber type genes in growing-finishing pigs. According to the study results, 1-1.5% CLA supplementation is appropriate.

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