

Dietary conjugated linoleic acid and leucine improve pork intramuscular fat and meat quality*

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ABSTRACT

This study investigated which conjugated linoleic acid (CLA) and leucine (LEU) ratio optimized the improvement of pork quality when added to finishing pig diets. A total of 120 finishing pigs were randomly assigned to five treatments, including a control diet and a 2 (LEU 1.0 and 2.0%) by 2 (CLA 0.5 and 1.0%) factorial design. The animals had free access to the diets and water. The pigs were slaughtered at 125 kg body weight. Growth performance, carcass characteristics, meat quality, sensory evaluation and serum cholesterol levels were measured. Data for each measured factor were analysed using the GLM procedure in SAS (1996). Analytical results revealed the IMF percentage (1.24, 1.04, 1.66, 2.31 and 1.61% for TRT1 to TRT5, respectively) and tenderness (2.14, 2.27, 2.32, 2.57 and 2.44 for TRT1 to TRT5, respectively) of the loin eye muscle were significantly ($P < 0.05$) improved by the CLA 0.5% combined with LEU 2.0%. Other indicators (such as growth performance, meat quality, carcass measurements and serum cholesterol level) were not affected by the LEU and CLA supplementation.

KEY WORDS: leucine, conjugated linoleic acids, intramuscular fat, pig

INTRODUCTION

Pork quality is a composite of numerous factors such as appearance, flavour, marbling, tenderness and juiciness. Marbling ideally 2-4% intramuscular fat (IMF) improves pork flavour, tenderness and juiciness, and thus is a critical factor for consumers. CLA is a mixture of positional and geometric isomers of linoleic acid combined *via* two conjugated double bonds at various carbon positions in the

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fatty acid chain. Feeding pigs a diet supplemented with 1.0% conjugated linoleic acid (CLA) has been demonstrated to improve belly firmness (Eggert et al., 2001), and 2.0% CLA supplementation increases IMF fat (Dugan et al., 1999; Gatlin, et al., 2002) or marbling on loin muscles (Wiegand et al., 2002), and giving leucine (LEU) has been shown to increase intramuscular fat when added to finishing pig feed (Cisneros et al., 1996; Hyun et al., 2003).

Two primary isomers of CLA found in commercial preparations are *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. The effects of CLA on lipogenesis seems variable with species, and the mechanisms of action regarding CLA's effects on lipid accumulation in adipocytes are still not well known. It has been shown that the *trans*-10, *cis*-12 CLA isomer specifically decreases fatty acid uptake and utilization of exogenously derived fatty acids, thereby limiting substrate for TG synthesis. Mechanisms have been suggested which including *trans*-10, *cis*-12 CLA down-regulates the mRNA expression of PPAR γ as well as its target genes, fatty acid binding protein (aP2) and liver X receptor α (LXR α) (Granlund et al., 2003). *Trans*-10, *cis*-12 CLA inhibits porcine preadipocyte differentiation by a mechanism that involves the down-regulation of PPAR γ and SREBP-1c mRNA. This mechanism is independent of changes in C/EBP α mRNA abundance and may involve COUP-TF (Brandebourg and Hu, 2005). *Trans*-10, *cis*-12 CLA promotes adipocyte delipidation, which may result from ERK-dependent alterations in perilipin and ADRP gene expression, protein activation, and localization (Chung et al., 2005). In contrast the *cis*-9, *trans*-11 isomer of CLA fails to inhibit the proliferation and differentiation of pig preadipocytes, and it may even stimulate the differentiation of these cells (Ding et al., 2000; McNeel and Mersmann, 2003). Given that a stimulatory effect of CLA on adipogenesis seems at odds with the ability of CLA to decrease carcass adiposity, the effect of CLA on the hyperplasia of adipocytes in pigs remains controversial.

Leucine (LEU) has been shown to increase intramuscular fat when added to finishing pig feed (Cisneros et al., 1996; Hyun et al., 2003). Studies have demonstrated that high LEU diet reduced pig growth performance; however, conflicting findings were acquired when the LEU level in pig diets was 2-6% (Edmonds and Baker, 1987; Hyun et al., 2003). Both CLA and LEU increased IMF. Whether different combinations of CLA and LEU applied to pig diet have a booster affect on pork IMF and quality warrants further investigation. This study elucidates the influence on pork quality when finishing diets are supplemented with a combination of CLA and LEU.

MATERIAL AND METHODS

Animal and experimental treatment

A total of 120 finishing pigs (Duroc × Landrace × Yorkshire; initial body weight, 60 kg) were randomly assigned to the following five treatments, including a control (without supplementing LEU or CLA), and a 2 (LEU 1.0 and 2.0%) by 2 (CLA 0.5 and 1.0%) factorial design. The CLA (BASF Chemical Company, France; purity 64%) comprised two major CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA). The LEU employed was a form of L-leucine (Ajinomoto Co., Inc. Japan), with purity of 98.5%. All five diets had similar nutrient composition, with the exception of LEU and CLA levels. Table 1 lists the diet formulation

Table 1. Diet formulation and nutrient composition of five treatments

Diets	Control ¹	LEU1.0+ CLA 0.5	LEU1.0+ CLA 1.0	LEU2.0+ CLA 0.5	LEU2.0+ CLA 1.0
<i>Ingredients, %</i>					
maize, dent, yellow	66.9	66.4	65.9	66.4	66.2
soyabean meal, solvent (44%)	22.0	21.0	21.0	19.0	19.0
wheat bran	5.0	5.0	5.0	5.0	5.0
molasses	2.0	2.0	2.0	2.0	2.0
tallow	1.0	1.0	1.0	2.0	2.7
limestone	1.2	1.2	1.2	1.2	1.2
dicalcium phosphate	1.15	1.15	1.15	1.15	1.15
salt	0.5	0.5	0.5	0.5	0.5
lysine-HCl	0.08	0.08	0.08	0.10	0.10
vitamins ²	0.1	0.1	0.1	0.1	0.1
minerals ³	0.1	0.1	0.1	0.1	0.1
l-leucine	0	1.0	1.0	2.0	2.0
conjugated linoleic acids	0	0.5	1.0	0.5	1.0
<i>Nutrient composition, calculated</i>					
crude protein, %	15.5	15.7	15.7	15.6	15.6
digestible energy, kcal/kg	3350	3335	3355	3342	3350
calcium, %	0.80	0.80	0.80	0.79	0.79
phosphorus, %	0.57	0.57	0.57	0.56	0.56
lysine, %	0.70	0.67	0.67	0.67	0.67

¹ control group with the diet formulation based on maize and soyabean meal without supplementing CLA or LEU

² supplied the following vitamin per ton of mixed diet: IU: vit. A 4,000,000; vit. D₃ 800,000; mg: vit. B₁ 1,250; vit. B₂ 2,000; vit. B₆ 300; vit. B₁₂ 10; pantothenic acid 6,000; niacin 15,000; folic acid 100; vit. E 10,000; vit. K₃ 1,250; choline chloride 50,000

³ supplied the following minerals per ton of mixed diet: mg: Cu 5,000; Fe 25,000; Mn 15,000; I 500; Zn 50,000

and nutrient composition of the five treatments. In this trial, four pigs were reared per pen with six pens (24 pigs) for one treatment. Feed and water were given *ad libitum*. The pig's body weight and average feed intake of each pen of four pigs were measured at 2-week intervals to assess growth performance. Growth performance was measured based on average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR). The trial ended when the pig reached 125 kg body weight.

Serum cholesterol analysis

Ten ml of blood was drawn from each pig on the final day of the trial. Following coagulation for one h at room temperature, blood clots were centrifuged (2500 g for 20 min at 4°C). Serum was then removed and stored at -20°C for subsequent measurement of serum cholesterol concentrations, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL) and triglycerides (TAG), which were performed using enzymatic methods and commercially available kits (VITRO Cholesterol kit, Ortho-Clinical Diagnostics, Johnson and Johnson Company, USA) suited to routine analysis. Very-low-density lipoprotein cholesterol (VLDL) content was derived by dividing TAG by 5 (Friedewald et al., 1972), and low-density lipoprotein cholesterol (LDL) levels were obtained by subtracting HDL and VLDL concentrations from TC.

Carcass measurements and meat quality determination

Carcass measurements including average back fat, carcass length and loin eye area (LMA) were taken once from each hog at 125 kg body weight. The LMA was measured at the 10th rib of the carcass. Average back fat was the average of measurements at three points: the first rib, the last rib, and, the last lumbar vertebra. Carcass length was measured from the first rib to the end of the pubic bone.

Meat quality was estimated using IMF percentage, marbling score, water holding capacity (WHC), pH value and loin muscle colour. Intramuscular fat measurements were performed *via* the following methods. Fat was measured by hexane extraction using the Soxhlet method (AOAC, 1990). pH value was measured at room temperature using a pH meter (PH330/SET-2, Wissenschaftlich-Technische Werkstätten, Germany) 12 h after slaughter. Marbling was measured at the 10th and 11th rib faces using the NPPC 6-point scale (NPPC, 1991). WHC was estimated using the Carver Press method, which utilizes a 1 g meat sample pressed at 3,000 psi for 3 min on a 125-mm filter paper to generate two distinct areas: a meat area; and, a water area. The WHC is the ratio of water area to meat area. Low ratio, which is desirable, indicates high water retention ability. pH

was measured as the mean from three points, with the measurements being taken directly from a loin chop using a Mettler Toledo electrode (Inlab 427). Finally, colour was measured using the Hunter Lab Color system (MiniScan XE Plus/Color Flex, Hunter Lab, USA) with a 2.5 cm objective.

Evaluation of sensory characteristics

Sensory characteristics were assessed for chops from loin eye muscle cooked in a boiler set at 90-95°C. Each chop was cut into 1.8 cm cubes for sensory evaluation. A panel comprising five to seven subjects evaluated the meat for tenderness, flavour and juiciness using a 5-point scale.

Statistical analysis

Effects of treatment on growth performance, carcass characteristic, meat quality, sensory evaluation and serum cholesterol level were analysed with the GLM procedure of SAS Institute (SAS, 1990). Initially, treatment effects were separated by employing single degree of freedom orthogonal contrasts to compare the control and treatment groups, and to compare LEU 1.0 vs 2.0% or CLA 0.5 vs 1.0% and the examine interactions between LEU and CLA. Since the interactions of LEU and CLA were not statistically significant, the effects of treatments was analysed by employing the GLM procedure of SAS as a repeated measure two-way ANOVA. When ANOVA revealed a significant effect, the differences, among treatment groups were tested using Duncan's new multiple range test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Table 2 lists the analytical results found for the five treatments on meat quality and sensory evaluation. The experimental results for growth performance, carcass measurements and serum cholesterol concentrations are given in Table 3. The IMF percentage and tenderness of loin eye muscle were higher for the CLA combined with LEU groups (TRT 3, 4 and 5) than for TRT 1, and particularly for TRT 4 (2.31%), which had a significantly higher ($P<0.05$) IMF percentage than TRT 1 (1.24%). Meanwhile WHC, pH, colour, juiciness and flavour of loin eye muscle were not significantly influenced by the addition of CLA and LEU. The groups fed CLA and LEU tends to have lighter loin eye muscle colour, and these groups also tender to have higher IMF percentage. Increased fat, typically reflected in a light colour, was associated with increased tenderness.

Table 2. Effects of CLA and LEU combination on meat quality and sensory evaluation

Treatment	CTL ¹	LEU1.0+ CLA0.5	LEU1.0+ CLA0.5	LEU1.0+ CLA 0.5	LEU1.0+ CLA 0.5	SE	Significance
No. of observation	24	24	24	24	24	-	-
<i>Meat quality</i>							
IMF, % ²	1.24	1.04 ^b	1.66 ^{ab}	2.31 ^a	1.61 ^{ab}	0.58	*
WHC ³	1.45	1.40	1.39	1.41	1.40	0.05	NS
pH value	5.54	5.58	5.53	5.53	5.57	0.02	NS
colour	3.56	3.00	3.00	2.75	3.38	0.53	NS
<i>Sensory evaluation</i>							
tenderness	2.14 ^b	2.27 ^{ab}	2.32 ^{ab}	2.57 ^a	2.44 ^{ab}	0.06	*
juiciness	1.90	2.13	2.05	2.17	2.05	0.05	NS
flavour	2.36	2.19	2.43	2.54	2.51	0.05	NS

¹ CTL - control group with the diet formulation based on maize and soyabean meal without supplementing CLA or LEU

² IMF - intramuscular fat percentage

³ WHC - water holding capacity

* means within the same row with different superscripts differ significantly (P<0.05); NS - P>0.05

Table 3. Effects of CLA and LEU combination on growth and carcass measurements of pigs and serum cholesterol concentration

Treatment	CTL ¹	LEU1.0+ CLA0.5	LEU1.0+ CLA0.5	LEU1.0+ CLA0.5	LEU1.0+ CLA0.5	SE	Significance
No. of pigs	24	24	24	24	24	-	-
<i>Growth performances</i>							
daily gain, g	794	697	726	720	738	17.3	NS
feed intake, kg/d ²	2.59	2.27	2.27	2.42	2.30	0.04	NS
feed/gain ²	3.26	3.40	3.20	3.14	3.12	0.14	NS
<i>Carcass measurements</i>							
carcass length, cm	91	93	92	92	92	5	NS
average backfat, cm	2.86	2.49	2.63	2.87	2.66	0.20	NS
loin eye area, in ²	8.19	7.88	8.44	7.14	8.68	3.36	NS
<i>Serum cholesterol, mg/dl</i>							
total cholesterol	74.8	76.0	82.0	88.7	71.3	2.7	NS
high-density lipoprotein	16.3	15.8	17.5	15.5	15.0	0.8	NS
low-density lipoprotein	51.5	56.3	58.8	62.0	51.0	2.1	NS
triglycerides	35.5	20.0	28.3	36.3	27.0	3.4	NS

NS - P>0.05

¹ see Table 1

² only six data were used in each treatment due to four pigs in each pen

Tenderness and IMF percentage were higher ($P < 0.05$) for TRT 4 compared to TRT 1. The juiciness and flavour of TRT 4 also tended to exceed that of the CTL, revealing that the best combination in terms of improving meat quality is 2.0% LEU and 0.5% CLA (TRT 4). The combination of LEU and CLA fed to group TRT 4 (2.0% of LEU and 0.5% CLA) obtained the best results overall. Feeding pigs a diet with 1.0% CLA has been demonstrated to increase bell firmness (Eggert et al., 2001) while a diet supplemented with 2.0% CLA increase IMF percentage (Dungan et al., 1999; Gatlin et al., 2002). Meanwhile, study has also showed that diets supplemented with 2.0% leucine increased ($P < 0.05$) longissimus fat content and marbling scores in finishing pigs (Cisneros et al., 1996; Hyun et al., 2003). CLA or LEU alone has been shown to improve IMF. This study examined whether supplementing diet with a combination of CLA and LEU may have an additive effect when used in an appropriate ratio. In this work, when diets were supplemented with 0.5% CLA, increasing the LEU levels from 1.0 (TRT 2) to 2.0% (TRT 4) increased the IMF percentage significantly ($P < 0.05$) from 1.04 to 2.31%, respectively, significantly ($P < 0.05$) exceeding that of the control group (1.24%). This experimental result is identical to that obtained by Hyun et al. (2003). Pork tenderness displayed a similar tendency, with TRT4 (2.57) significantly ($P < 0.05$) exceeding that of TRT 1 (2.14). However, when 1.0% CLA was added to the diets and the LEU level was increased from 1.0 (TRT 3) to 2.0% (TRT 5), IMF and other criteria failed to improve, indicating that high CLA (1.0%) yielded the best results when combined with low LEU (1%). Other combinations of CLA and LEU also improved meat flavour, juiciness and tenderness, but the improvements were not statistically significant.

The effect of CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA) has been variable and occurs by an unknown mechanism (Azain et al., 2003; Dugan et al., 2004). Although hyperplasia of human primary preadipocytes is potently inhibited *in vitro* by the *trans*-10, *cis*-12 CLA isomer (Brown et al., 2003), it has been reported that *cis*-9, *trans*-11 CLA fails to inhibit the proliferation and differentiation of pig preadipocytes, and it may even stimulate the differentiation of these cells (Ding et al., 2000; McNeel and Mersmann, 2003). In this trial, the significantly increase of IMF percentage suggested by the boosting effect of *cis*-9, *trans*-11 CLA combined with leucine, but the effect of *trans*-10, *cis*-12 CLA in this case is still hard to understand and need more study to clarified.

One and 2% leucine (TRT 2 to TRT 5) were used in this study. High percentages of leucine in pig diets have been shown to retard pig growth rate in some studies (Cisneros et al., 1996; Hyun et al., 2003). However, in this study, 1.0–2.0% of leucine supplementation in finishing pig diets did not significantly reduce growth rate or feed intake. This result is different from those obtained by Taylor et al. (1984), Cisneros et al. (1996) and Hyun et al. (2003), in which 0.7 and 2.0% leucine reduced pig growth performance. However, Edmonds and Baker (1987)

demonstrated that pig growth performance was only hindered with diets containing 6% leucine, but not with lower levels of supplementation. This discrepancy in experimental results may result from difference in feeding intervals, starting age, body weight or health status of the experimental pigs; for example, Edmonds and Baker (1987) used 4-week-old weaning pigs (8 kg BW), whereas the other three studies used, hogs weighing 25-115 kg. Whether body weight is the primary reason for the different results requires further study.

Regarding loin muscle colour, muscle colour ($P < 0.01$) was darker when finishing pig diet was supplemented with 2.0% LEU (Cisneros et al., 1996). However, Hyun et al. (1996) found no influence on loin muscle colour for a diet supplemented with 2.0% LEU. Joo et al. (2002) and Corino et al. (2003), both of whom supplemented pig diets with 0.25-5.0% CLA, demonstrated that the colour of loin eye muscle, fat tissue and intramuscular fat did not differ from that in the CTL group. In this work, the finishing pigs eating diets supplemented with both LEU and CLA tended to have lighter loin muscle colour than the control group. Whether CLA has lightens muscle colour thus requires further investigations.

This study found no impact on carcass measurements (carcass length, average back fat and loin eye area) and serum cholesterol (TC, HDL, LDL and TAG) from dietary supplementation with LEU and CLA. Although some studies in experimental animals (hamsters) found a significant cholesterol-lowering effect when fed the *trans*-10, *cis*-12 isomer, but not when they were fed the *cis*-9, *trans*-11 isomer (de Deckere et al., 1999; Gavino et al., 2000). Most of the numerous studies in experimental animals such as mice, rats, and pigs did not show any effect on plasma cholesterol concentrations. This finding resembles the findings of Taylor et al. (1984), Cisneros et al. (1996), Gatlin et al. (2002) and Hyun et al. (2003), involving LEU or CLA supplementation ranging from 0.7-2.0% in pig diets. However, these results differ from those of Sun et al. (2004), in which supplementation with 0.125-4.0% CLA improved loin eye area and reduced back fat thickness.

CONCLUSIONS

Supplementing LEU and CLA in finishing pig diets improved pork quality (IMF and tenderness) and taste, especially when 0.5% CLA was combined with 2.0% LEU (TRT 4), and significantly ($P < 0.05$) increased intramuscular fat levels of the longissimus muscle thus improving muscle tenderness. The significantly increase of IMF percentage suggested by the boosting effect of *cis*-9, *trans*-11 CLA combined with leucine that stimulate the proliferation and differentiation of pig preadipocytes. However, supplementation with CLA and LEU appeared not to affect growth performance, carcass measurement, and serum cholesterol concentrations.

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