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Effect of CLA Supplementation to Low-Protein Diets on the Growth Performance, Carcass Characteristics, Plasma Urea Nitrogen Concentration, and Fatty Acid Profile in the Meat of Pigs

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ABSTRACT

To analyze the effect of conjugated linoleic acid (CLA) on the meat of pigs (0,1%) and three crude protein (CP) levels (nursery: 20.5, 16.0, 14.5%; growing: 16, 14.5, 11.5%; and finishing: 14.0, 12.5, 11% CP), studies were conducted with 36 hybrid (Yorkshire×Landrace×Duroc) barrows (17.3-83.5 kg), which were individually penned and allotted in a completely randomized design in a factorial (2×3) arrangement for 84 d. The analysis by phases indicated that CP level affected some variables. Average daily gain, average daily feed intake, fat free lean gain, backfat thickness, longissimus muscle area and final body weight were reduced (P≤0.05) feeding the lowest CP diet in nursery and growing pigs. Plasma urea nitrogen concentration was also lower (P≤0.05) in the growing and finishing phases when fed the lowest CP level. The global analysis showed that all the analyzed variables (except feed gain ratio, lean meat percentage and plasma urea nitrogen concentration) were reduced (P≤0.05) in the pigs fed low-protein diets; plasma urea nitrogen concentration tended to be lower (P=0.07) when CP was reduced. The fatty acid profile of the meat (semimembranosus and longissimus muscles) indicated that CLA addition increased CLA isomers and total saturated fatty acids, and reduced the total monounsaturated fatty acids (P≤0.05). α-Linolenic acid was lowered in longissimus muscle of pigs fed LPD (P=0.08). These results indicated that reducing the crude protein concentration in the diet of fattening pigs from 20.5 to 16.0% in nursery phase; from 16.0 to 14.5% in growing stage; and from 14.0 to 12.5% in finishing pigs, did not negatively affect the growth performance, nor carcass characteristics. The results also showed that the addition of CLA did not improve pig response and the concentration of unsaturated fatty acids and total lipids altered the feeding LPD.

Key words: Pigs, Low-protein diets, Conjugated linoleic acid, Fatty acid profile in meat

INTRODUCTION

The reduction of crude protein (CP) in sorghum-soybean meal diets, up to 4.0%, properly supplemented with crystalline amino acids (AA), does not adversely affect weight gain or feed efficiency of pigs; in addition, it helps to reduce nitrogen (N) excretion in feces and urine (Canh et al. 1998; Kerr et al. 2003; Shriver et al. 2003). It also reduces the energy expenditure associated with the excretion of excess of dietary CP as urea and lowers the metabolic heat production when higher levels of CP are fed. However, this energy is then available for the synthesis of lipids (Le

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Bellego et al. 2002), leading to an increased accumulation of fat in the carcass of pigs fed low-protein diets (LPD) (Knowles et al. 1998). This is a negative factor because the consumer demands leaner pork and better marbling for human consumption.

The addition of conjugated linoleic acid (CLA) to the diet can help to reduce the fatness in pig carcass. CLA is formed by a group of positional and geometric isomers of linoleic acid, an essential fatty acid of the omega-6 family (Jensen 2002). Studies in rodents have shown that CLA reduces fat deposition and increases the synthesis of lean tissue (Park et al. 1997; 1999). There may be several mechanisms involved in this, although the more accepted are that CLA increases the energy expenditure, regulates the adipocyte metabolism, regulates adipokines and cytokines, increases the β-oxidation in skeletal muscle (Park and Pariza 2007) and decreases the catabolic effect of immune function in muscle (Pariza et al. 2000). In pigs, it was observed that the incorporation of CLA in the diet improved the growth performance and carcass characteristics (Thiel-Cooper et al. 2001; Wiegand et al. 2001; Su et al. 2006), and modified the type and concentration of other fatty acids, which could improve the processing of meat (King et al. 2004). In addition, it increased the concentration of CLA in the meat (Wiegand et al. 2002; Lauridsen et al. 2005; Schmid et al. 2006), which might have benefits for human nutrition and health through preventive and therapeutic properties in the diseases such as cancer, chronic inflammation, atherosclerosis, obesity and antioxidant function (Roche et al. 2001).

The objective of this study was to evaluate the effect of CLA addition replacing soybean oil, to low-protein, sorghum-soybean meal diets fed to the fattening pigs on the growth performance, carcass characteristics, plasma urea nitrogen concentration, and fatty acid profile and concentration in the meat of *longissimus* and *semimembranosus* muscles.

MATERIALS AND METHODS

Pigs and experimental design

Thirty six hybrid (Yorkshire×Landrace×Duroc) barrows with 17.3±2.0 kg of body weight were used. These were distributed in a completely randomized design with factorial (2×3) arrangement at two levels of CLA and three levels of CP, with six replicates per treatment during 21, 28, and 35 days in nursery, growing, and finishing phases, respectively.

Diets and general management of pigs

The diets were based on sorghum-soybean meal and were formulated based on true digestible amino acids (NRC 1998) to meet or exceed the nutritional requirement for each stage of the growth of pigs (Table 1). The CP concentrations evaluated for each phase (nursery: 20.5, 16.0, and 14.5%; growing: 16.0, 14.5, and 11.5%; finishing: 14.0, 12.5, and 11.0%) were as follows: control level (standard diet; first concentration); the second level (low-protein) corresponded to the CP content in the diet where growth performance was similar to that obtained with the standard CP level, and the third level was the CP concentration with the lowest plasma urea nitrogen concentration in the nursery (Trujillo-Coutiño et al. 2007), growing (Martínez-Aispuro et al. 2009), and finishing (Figueroa et al. 2008) pigs. Dietary supplementation of the CLA was 0 or 1.0%, replacing soybean oil in the diet (Table 2). The barrows were individually housed in 1.2×1.5 m pens with concrete floor, equipped with a single feeder and a nipple drinker. Feed and water were provided *ad libitum*.

Data recording, sampling and laboratory analysis

The change of body weight to determine average daily gain (ADG), as well as feed disappearance to estimate the average daily feed intake (ADFI) and feed: gain ratio (FGR) were registered on the first and last day of each stage. Blood samples were collected on the first and last day of each stage using vacutainer heparinized tubes (BD Vacutainer Systems, NJ, USA). The blood was centrifuged at 1286 *g* during 15 min and the supernatant was transferred to polypropylene tubes and stored at -20°C (EUR251P7W Tappan, Electrolux Home Products North America, USA) until laboratory determination of plasma urea nitrogen concentration (PUN; Chaney and Marbach 1962). On the first and last day of each stage the backfat thickness (BT) and *longissimus* muscle area (LMA) were also measured using a real time ultrasound Sonovet 600 with a 3.5 MHz transducer (Medison, Inc., Cypress, California, USA). These data together with the initial and final weights were used to determine the fat free

lean gain (FFLG) and the lean meat percentage (LMP) using the NPPC (1991) equation. Crude protein (CP) was determined in feed samples (AOAC 1990).

Pigs were slaughtered in a commercial abattoir. Animals were stunned in a V-type restraining conveyor using a high-voltage electric apparatus. Pigs were bled in a lying position, and generally were stuck within 5 s after stunning. Afterward, they were eviscerated and scaled, preserving the carcass from the skin, head and limbs. Later, carcasses were transported to a cutting room. Meat samples from *semimembranosus* (SM) and *longissimus* (LM) muscles were collected from the warm carcasses of pigs. There was a fasting period 12 h before the slaughter. There was no opportunity of measuring the carcass characteristics at this place because it was no allowed. Meat samples were macerated with a food processor and frozen at -20°C (EUR251P7W Tappan, Electrolux Home Products North America, USA) until the determination of total concentration of fatty acids (saturated, unsaturated, polyunsaturated, and CLA isomers) in muscle tissues, dietary oil and CLA sources.

Table 1 - Composition of experimental diets for fattening pigs on an air-dry weight basis (g/kg).

Phase	**Nursery**			**Growing**			**Finishing**		
Crude protein	**205**	**160**	**145**	**160**	**145**	**115**	**140**	**125**	**110**
Ingredient/Treatment‡	**T1**‡	**T3**‡	**T5**‡	**T1**‡	**T3**‡	**T5**‡	**T1**‡	**T3**‡	**T5**‡
Sorghum grain	622.55	765.88	813.66	760.55	808.34	903.91	822.55	870.27	918.06
Soybean meal (44%)	333.46	183.35	133.29	197.08	147.02	46.90	140.72	90.75	40.69
Soybean oil	15.91	11.41	9.91	14.57	13.07	10.07	13.16	11.63	10.13
Bio-Lys (L-Lisina·H_2SO_4)-*	0.93	3.04	3.75	3.69	4.40	5.81	3.14	4.03	4.73
DL-Methionine	0.17	1.40	1.81	0.22	0.63	1.45	0.00	0.40	0.81
Tripto-Plus (L-Tryptophan) **	0.22	4.65	6.13	0.20	1.68	4.64	0.00	1.31	2.79
L-Threonine	0.00	1.83	2.45	0.31	0.93	2.17	0.08	0.70	1.31
Vitamins-Minerals premix ***	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Salt	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Antioxidant (Etoxiquine)	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
$CaCO_3$	10.25	10.93	11.15	10.27	10.50	10.95	8.80	9.02	9.25
Conjugated linoleic acid ****	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Dicalcium phosphate	10.28	11.28	11.62	6.87	7.21	7.88	5.33	5.67	6.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Analysis, (g/kg)									
Metabolizable energy, Mcal kg^{-1}	3.279	3.279	3.279	3.308	3.308	3.308	3.319	3.319	3.319
Crude protein	205.0	160.0	145.0	160.0	145.0	115.0	140.0	125.0	110.0
Calcium	7.0	7.0	7.0	6.0	6.0	6.0	5.0	5.0	5.0
Available phosphorus	3.2	3.2	3.2	2.3	2.3	2.3	1.9	1.9	1.9
Lysine	10.1	10.1	10.1	8.3	8.3	8.3	6.6	6.6	6.6
Threonine	6.5	6.5	6.5	5.2	5.2	5.2	4.3	4.3	4.3
Tryptophan	2.3	2.3	2.3	1.7	1.7	1.7	1.4	1.4	1.4
Methionine	3.0	3.6	3.8	2.5	2.7	3.1	2.0	2.2	2.4
Arginine	12.1	8.1	6.7	8.5	7.1	4.4	7.0	5.6	4.3
Histidine	4.7	3.4	2.9	3.5	3.1	2.2	3.0	2.6	2.1
Isoleucine	7.8	5.7	5.0	5.9	5.2	3.8	5.1	4.4	3.7
Leucine	16.8	13.9	12.9	14.2	13.3	11.3	13.2	12.2	11.3
Valine	8.4	6.4	5.7	6.6	5.9	4.5	5.8	5.1	4.4
Methonine + Cystine	5.8	5.8	5.8	4.7	4.7	4.7	4.0	4.0	4.0
Determined anaylsis, %									
Crude protein	212.0	173.0	152.0	166.0	155.0	120.0	135.0	119.0	113.0

‡T1, T3, T5 are the basal diets without CLA addition.

* BioLys contains: crude protein, 75%; available phosphorus, 0.16%; lysine, 50.7%; threonine, 0.4%; tryptophan, 0.14%; methionine, 0.2%; arginine, 0.6%; isoleucine, 0.4%; leucine, 0.7%; valine, 0.7%; cystine, 0.1%.

* Tripto Plus contains: crude protein, 95%; lysine, 55.3%; threonine, 0.15%; tryptophan, 15%; valine, 0.5%; methionine+cystine, 1.75%.

** Each kg of feed supplied: vitamin A, 6250 IU; vitamin D, 1250 IU; vitamin E, 25 IU; vitamin K3, 2.5 mg; B1, 1.25 mg; B2, 6.25 g; B5, 31.25 mg; B6, 2.5 mg; B12, 0.01875 mg; folic acid, 3.75 mg; Vit. H, 0.225 mg; pantothenic acid, 18.75 mg; choline, 381.25 mg; Fe, 125 mg; Zn, 125 mg; Mn, 125 mg; Cu, 12.5 mg; Se, 0.25 mg; I, 0.375 mg; Co, 0.125 mg.

**** Conjugated linoleic acid (LutaCLA® 60 BASF Mexicana) contains: 9c, 11t metyl ester, 30%; 10t, 12c metyl ester, 30%; other isomers, ≤1%; oleic acid, 22%; palmytic acid, 6%; stearic acid, 4%; linoleic acid, 2%; methanol, ≤100 ppm; heavy minerals, ≤1 ppm.

Table 2 - Fatty acid profile in CLA and soybean oil used in the experimental diets (g/kg).

Fatty acid, % FAME's*	CLA	CSO
Palmitic (C16:0)	51.1	10.32
Palmitoleic (C16:1)	ND	0.10
Heptadecanoic (C17:0)	ND	0.10
Stearic (C18:0)	42.6	4.16
Oleic (C18:1)	229.5	20.89
Cis-vaccenic (C18:1)	ND	0.96
Linoleic (C18:2)	4.6	54.94
Alfa-linolenic (C18:3)	ND	7.50
c9,t11 y c11,t9 CLA	323.9	ND
t10,c12 CLA	300.0	ND
Arachidonic (C20:4)	ND	0.32
Eicosaenoic (C20:1)	5.5	0.20
Eicosapentaenoic (C20:5)	6.6	0.34
Erucic (C22:1)	8.2	ND
Lignoceric (C24:0)	1.4	0.11
Other fatty acid	26.5	0.08
Saturated fatty acids	95.1	15.00
Monounsaturated fatty acids	243.2	22.14
Polyunsaturated fatty acids	635.1	62.78

*FAME's = fatty acid methyl esters; CLA = conjugated linoleic acid; CSO = crude soybean oil; ND = not detectable.

Samples were processed according to the methods described by Folch for total lipid analysis (Folch et al. 1957). Methyl esters of meat fatty acids were obtained and saponification was performed using boron trifluoride. Fatty acids were quantified by gas chromatography using a DB-23 column (JW 122-2332 of 30 m × 0.25 mm internal diameter) on a Varian 3400 CX gas-liquid chromatograph, equipped with an autosampler and a flame ionization detector (Varian Associates, Inc., Sugar Land, TX). Myristic acid (Sigma Chemical Co., St. Louis, MO.) was used as an internal standard for fatty acids (method 994.10; AOAC 2000). Retention times were compared with fatty acid methyl ester standards.

Statistical analysis

The ADG, ADFI, and FGR were analyzed using the average of each stage of the growth with the general linear model (GLM) procedure of SAS (2002) and the statistical models indicated. Initial body weight was used as a covariate in the statistical analysis of the variables that required it. The means treatment comparison of the main factors effect was performed with the Tukey test or LSMEANS ($P \leq 0.07$).

RESULTS

Nursery phase

No interaction between the CLA and CP level was observed on the growth response and carcass characteristics variables (Table 3), or on PUN concentration ($P>0.05$). The reduction of CP by 6.0% diminished ADG and final weight (FW) and ADFI ($P \leq 0.05$) without affecting the FGR ($P>0.05$). But, lower BT, LMA, and FFLG ($P \leq 0.01$) were observed. However, pigs fed 16.0% CP had higher BT and LMA ($P \leq 0.05$), although similar FFLG as in the pigs fed 20.5% CP. The final LMP and PUN concentration were not affected ($P>0.05$) by the dietary CP level. Supplementation of the CLA in the diet did not improve ($P>0.05$) growth performance, carcass characteristics or PUN of the pigs.

Growing phase

There was no interaction ($P>0.05$) between the main factors (Table 4). Reducing dietary CP from 16.0 to 11.5% decreased final BW, ADG and ADFI ($P \leq 0.05$), but not FGR ($P>0.05$). When CP was lowered from 16.0 to 14.5%, the FFLG was similar; however, when CP was reduced up to 11.5%, FFLG was 58 g d^{-1} ($P \leq 0.05$) and LMA was 419 mm^2 lower ($P \leq 0.05$) than in the pigs fed 16.0% CP. The BT and LMP were not affected by the dietary CP level ($P>0.05$). The PUN was 39% lower in the pigs fed 11.5% CP ($P \leq 0.05$). The CLA supplementation had no effect ($P>0.05$) on the analyzed variables.

Finishing phase

The reduction of CP level from 14.0 to 11.0% tended to diminish the LMA ($P=0.07$) and lowered PUN concentration ($P \leq 0.05$) (Table 5). However, there was no effect of main factors or their interaction ($P>0.05$) on other variables in this stage of growth.

Whole fattening period

Because CLA was supplemented throughout the fattening period (nursery, growing and finishing stages) to the same pigs in each treatment, an overall statistical analysis was performed to detect the probable effects in the whole trial. This analysis (Table 6) showed that CLA supplementation and the interaction of main factors did not affect the variables under the study. The CP level affected ($P \leq 0.05$) the ADG and final BW, which were similar in the pigs fed standard and intermediate level of CP. However, pigs fed the lowest CP level showed 12 kg less BW, 144 g d^{-1} less ADG, and lower ADFI and FFLG ($P \leq 0.05$). The FGR was not affected by the dietary CP level ($P>0.05$). The *longissimus* muscle area and backfat thickness (Table 6) were reduced in

the pigs fed the lowest dietary CP (P≤0.05). The LMP was not affected by the dietary CP concentration (P>0.05). Plasma urea nitrogen concentration tended to be reduced in the pigs fed the lowest CP level (P=0.07).

Table 3 - Effect of dietary crude protein and linoleic acid concentration on growth performance, carcass characteristics and plasma urea nitrogen concentration of nursery pigs*.

			Growth performance						Carcass characteristics			
TRT	CP (gkg^{-1})	CLA (gkg^{-1})	BWi kg	BWf kg	ADG g d^{-1}	ADFI g d^{-1}	FGR	FFLG g d^{-1}	BT mm	LMA mm^2	LMP	PUN mg 100 mL
1	205	0	17.3	33.3	757	1.53	2.03	309	3.50	1375	44.4	6.19
2	205	10	17.4	32.8	732	1.50	2.05	289	3.33	1337	44.4	5.01
3	160	0	17.2	33.0	745	1.58	2.13	307	4.01	1441	44.7	4.12
4	160	10	17.7	33.8	783	1.63	2.09	300	3.62	1384	44.2	5.35
5	145	0	17.4	31.0	649	1.41	2.17	269	2.99	1261	44.9	4.44
6	145	10	17.2	30.3	617	1.31	2.13	251	3.02	1224	44.8	4.19
SEM				0.242	0.011	0.027	0.028	0.001	0.079	19.18	0.222	0.325
Main effects												
	205		17.3	33.0a	745a	1.52ab	2.04	299a	3.41ab	1356ab	44.4	5.60
	160		17.4	33.4a	764a	1.60a	2.11	303a	3.82a	1413a	44.5	4.73
	145		17.3	30.7b	633b	1.37b	2.15	260b	3.01b	1243b	44.9	4.30
		0	17.3	32.5	717	1.51	2.10	295	3.50	1359	44.7	4.94
		10	17.4	32.3	710	1.48	2.09	280	3.32	1315	44.5	4.85
Source of variation									***P* value**			
CP				0.001	0.001	0.004	0.285	0.002	0.001	0.004	0.714	0.278
CLA				0.783	0.784	0.658	0.776	0.146	0.273	0.261	0.639	0.923
CP × CLA				0.404	0.402	0.534	0.861	0.859	0.575	0.969	0.883	0.316
BWi **				0.001	0.001	0.005		0.018	0.006	0.001	0.005	

[a, b] Treatment means or main effect with different superscript by row, differ (P≤0.05).

* TRT = treatment; CP = crude protein; CLA = conjugated linoleic acid; SEM = standard error of the mean; BWi = initial body weight; BWf = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FGR = feed: gain ratio; FFLG = fat free lean gain; BT = backfat thickness; LMA = *longissimus* muscle area; LMP = lean meat percentage; PUN = plasma urea nitrogen concentration. ** Treatment means adjusted by initial body weight as covariate (P≤0.05).

Table 4 - Effect of dietary crude protein and conjugated linoleic acid concentration on growth performance, carcass characteristics, and plasma urea nitrogen concentration of growing pigs*.

			Growth performance						Carcass characteristics			
TRT	CP (gkg^{-1})	CLA (gkg^{-1})	BWi kg	BWf Kg	ADG g d^{-1}	ADFI g d^{-1}	FGR	FFLG g d^{-1}	BT mm	LMA mm^2	LMP	PUN mg 100 mL
1	160	0	33.3	55.5	823	2.20	2.67	324	6.23	2372	42.5	17.03
2	160	10	32.8	54.6	789	2.23	2.86	301	6.35	2220	41.9	16.50
3	145	0	32.8	54.2	775	2.21	2.88	308	5.95	2343	42.8	16.18
4	145	10	35.1	53.6	754	2.12	2.77	296	6.47	2396	42.7	14.38
5	115	0	31.0	50.7	653	1.89	2.97	234	5.97	1891	41.4	10.68
6	115	10	30.1	51.5	679	1.92	2.93	251	5.63	1878	41.5	9.81
SEM				0.386	0.013	0.040	0.056	0.001	0.132	50.40	0.244	0.327
Main effects												
	160		33.1	55.0a	806a	2.22a	2.75	313a	6.29	2303a	42.2	16.79a
	145		33.8	53.9a	764a	2.17ab	2.83	302a	6.21	2367a	42.8	15.36a
	115		30.5	51.1b	666b	1.90b	2.95	242b	5.80	1884b	41.4	10.20b
		0	32.4	53.5	750	2.10	2.83	289	6.05	2220	42.2	14.86
		10	32.5	53.2	741	2.09	2.85	283	6.15	2147	42.0	13.33
Source of variation									***P* Value**			
CP				0.002	0.002	0.017	0.418	0.002	0.348	0.001	0.145	0.001
CLA				0.738	0.734	0.893	0.927	0.690	0.721	0.717	0.693	0.115
CP × CLA				0.657	0.659	0.771	0.543	0.538	0.445	0.703	0.837	0.716
BWi **				0.001	0.001	0.002		0.006	0.010		0.002	

[a, b] Treatment or main effect means with different superscript by row, differ (P≤0.05).

* TRT = treatment; CP = crude protein; CLA = conjugated linoleic acid; SEM = standard error of the mean; BWi = initial body weight; BWf = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FGR = feed: gain ratio; FFLG = fat free lean gain; BT = backfat thickness; LMA = *longissimus* muscle area; LMP = lean meat percentage; PUN = plasma urea nitrogen concentration.

** Treatment means adjusted by initial body weight as covariate (P≤0.05).

Table 5 - Effect of crude protein and conjugated linoleic acid concentration on growth performance, carcass characteristics and plasma urea nitrogen concentration of finishing pigs*.

			Growth performance						Carcass characteristics			
TRT	CP (gkg^{-1})	CLA (gkg^{-1})	BWi kg	BWf kg	ADG gd^{-1}	ADFI gd^{-1}	FGR	FFLG g d^{-1}	BT mm	LMA mm^2	LMP	PUN mg 100 mL
1	140	0	56.8	84.1	885	2.80	3.17	293	10.85	3117	39.3	19.72
2	140	10	53.9	86.3	948	3.08	3.26	326	10.61	3015	38.9	19.20
3	125	0	56.1	84.5	896	2.94	3.29	284	10.47	3024	39.0	18.38
4	125	10	56.0	83.9	879	2.70	3.09	299	9.99	3114	39.6	15.35
5	110	0	48.3	81.7	817	2.55	3.11	287	10.03	2849	38.9	15.02
6	110	10	47.5	82.1	827	2.70	3.29	272	9.98	2691	38.2	14.36
	SEM			0.623	0.017	0.054	0.041	0.001	0.211	48.01	0.186	0.682
		Main effects										
	140		55.7	85.2	917	2.94	3.21	310	10.73	3066	39.1	19.51a
	125		56.1	84.2	887	2.82	3.18	292	10.23	3069	39.3	16.73ab
	110		47.9	81.9	822	2.63	3.21	279	10.00	2770	38.6	14.68a
		0	53.9	83.4	866	2.76	3.19	288	10.45	2996	39.1	17.83
		10	52.3	84.1	885	2.83	3.21	299	10.19	2940	38.9	15.94
		Source of variation								***P* Value**		
CP				0.194	0.195	0.156	0.964	0.315	0.447	0.072	0.336	0.033
CLA				0.607	0.609	0.563	0.768	0.441	0.555	0.567	0.578	0.318
CP × CLA				0.664	0.664	0.153	0.163	0.410	0.913	0.543	0.343	0.705
BWi**				0.001	0.011	0.005		0.011	0.001	0.001	0.024	

[a, b] Treatment or main effect means with different superscript by row, differ (P≤0.05).

* TRT = treatment; CP = crude protein; CLA = conjugated linoleic acid; SEM = standard error of the mean; BWi = initial body weight; BWf = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FGR = feed: gain ratio; FFLG = fat free lean gain; BT = backfat thickness; LMA = *longissimus* muscle area; LMP = lean meat percentage; PUN = plasma urea nitrogen concentration.

**Treatment means adjusted by initial body weight as covariate (P≤0.05).

Table 6 - Effect of crude protein and conjugated linoleic acid level on growth performance, carcass characteristics, and plasma urea nitrogen concentration of 17.3-83.5 kg pigs*.

			Growth performance						Carcass characteristics			
TRT	CP***	CLA (gkg^{-1})	BWi kg	BWf kg	ADG g d^{-1}	ADFI g d^{-1}	FGR	FFLG g d^{-1}	BT mm	LMA mm^2	LMP	PUN mg 100 mL
1	1	0	17.4	88.8	851	2.34	2.75	315	11.50	3252	39.0	19.7
2	1	10	16.8	85.6	812	2.38	2.95	294	10.20	2951	39.1	17.8
3	2	0	17.3	88.6	849	2.40	2.83	310	11.00	3134	38.8	18.4
4	2	10	17.9	88.4	846	2.32	2.75	314	11.00	3365	39.5	15.7
5	3	0	17.4	75.3	690	1.96	2.84	254	9.20	2674	39.3	15.0
6	3	10	17.2	75.0	687	1.99	2.92	246	9.00	2483	38.6	14.4
	SEM			1.216	0.014	0.044	0.031	0.001	0.275	56.65	0.203	0.713
		Main effects										
	1		17.1	87.2a	832a	2.36a	2.84	304[a]	10.90a	3115a	39.0	18.8
	2		17.6	88.5a	847a	2.36a	2.79	312[a]	11.00a	3249a	39.1	17.0
	3		17.3	75.1b	688b	1.97b	2.87	250b	9.09b	2570b	38.9	14.6
		0	17.4	84.2	797	2.23	2.80	293	10.62	3035	39.1	17.8
		10	17.3	83.0	782	2.23	2.87	285	10.00	2905	39.0	15.8
		Source of variation								***P* Value**		
CP				0.001	0.001	0.001	0.555	0.001	0.015	0.001	0.941	0.079
CLA				0.612	0.611	0.959	0.317	0.412	0.374	0.449	0.974	0.230
CP × CLA				0.838	0.840	0.820	0.194	0.639	0.589	0.158	0.419	0.842
BWi**				0.001	0.016	0.028		0.029				

[a, b] Treatment or main effect means with different superscript by row, differ (P≤0.05).

* TRT = treatment; CP = crude protein; CLA = conjugated linoleic acid; SEM = standard error of the mean; BWi = initial body weight; BWf = final body weight; ADG = average daily gain; ADFI = average daily feed intake; FGR = feed: gain ratio; FFLG = fat free lean gain; BT = backfat thickness; LMA = *longissimus* muscle area; LMP = lean meat percentage; PUN = plasma urea nitrogen concentration.

** Treatment means adjusted by initial body weight as covariate (P≤0.05).

***CP 1,2,3 means level of CP (1=standard; 2=middle; 3=low).

Fatty acids concentration in meat

There was no effect of interaction between the CP×CLA level on the fatty acid profile, except for linoleic acid (P≤0.045; Table 7) in the SM muscle and c9,t11 and c11,t9 CLA isomer (P≤0.039; Table 8) in the LM. Tables 7 and 8 present the fatty acid profile for the standard CP (control diet) and the lowest value of CP and CLA level.

Semimembranosus muscle

Total lipid concentration and fatty acids profile in *semimembranosus* muscle (SM; Table 7) were only affected (P≤0.05) by the dietary oil concentration. The level of CLA isomers in the SM was higher (P≤0.05) in the pigs fed 10 g CLA kg^{-1} of feed. Dietary CLA increased (P≤0.05) the myristic, palmitic, and palmitoleic acids, and reduced (P≤0.05) the oleic and linoleic acids. Total saturated fatty acid (SFA) concentration increased and the total monounsaturated fatty acid (MUFA) content decreased by the addition of CLA in the diet (P≤0.05).

Table 7 - Effect of crude protein and conjugated linoleic acid level on total lipids and fatty acids profile in *semimembranosus* muscle of pig.

	CP*		CLA concentration (%)			*P* value		
Fatty acids, % FAME´s	**Control**	**LPD**	**0.0**	**1.0**	**SEM**	**CP**	**CLA**	**CP×CLA**
Myristic (C14:0)	12.4	13.7	10.2b	15.9a	0.6	0.286	0.001	0.353
Cis 10-Pentanoic (C15:1)	6.5	8.9	7.8	7.6	1.4	0.426	0.949	0.909
Palmitic (C16:0)	241.1	246.8	229.2b	258.7a	61	0.676	0.045	0.402
Palmitelaidic (C16:1)	1.3	1.6	1.8	1.1	0.4	0.688	0.24	0.24
Palmitoleic C16:1	38.3	34.4	26.1b	46.6a	2	0.341	0.001	0.881
Heptadecanoic (C17:0)	1.8	2.0	2.1	1.7	0.3	0.75	0.488	0.915
Cis 10-heptadecenoic (C17:1)	5.4	2.4	2.1	5.6	1.9	0.416	0.364	0.329
Stearic (C18:0)	112.6	116.7	111.7	117.5	3.2	0.531	0.381	0.674
Elaidic (C18:1; n-9 *trans*)	6.5	3.1	2.8	6.8	1.9	0.367	0.314	0.254
Oleic (C18:1)	345.3	367.8	390.1a	323.1b	11	0.325	0.01	0.755
Cis-vaccenic (C18:1)	35.4	33.4	33.7	35.1	1.2	0.423	0.59	0.59
Linolelaidic (C18:2; n-6 *trans*)	0.7	0.5	0.3	0.9	0.2	0.644	0.13	0.554
Linoleic (C18:2; n-6)	101.7	97.1	112.2a	86.5b	4.5	0.624	0.015	0.045
c9,t11 and c11,t9 CLA	3.8	4.6	1.0b	7.4a	0.7	0.595	0.001	0.993
t10,c12 CLA	0.4	1.0	0.0b	1.4a	0.2	0.202	0.006	0.202
Other isomers of CLA	1.2	0.8	0.0b	1.4a	0.3	0.563	0.007	0.563
Gama-linolenic (C18:3)	0.2	0.4	0.5	0.1	0.2	0.689	0.312	0.863
Alfa-linolenic (C18:3; n-3)	3.0	3.6	3.9	2.6	0.4	0.51	0.123	0.723
Arachidic (C20:0)	1.3	2.1	1.7	1.6	0.3	0.172	0.89	0.391
Eicosaenoic (C20:1)	4.3	6.4	6.0	4.7	0.6	0.086	0.271	0.549
Cis-11, 14-eicosadienoic (C20:2)	2.8	2.9	3.2	2.5	0.4	0.965	0.427	0.411
Cis-11,14,17-eicosatrienoic (C20:3)	0.3	0.7	0.2	0.7	0.2	0.288	0.264	0.863
Cis-8,11,14-eicosatrienoic (C20:3)	1.9	1.9	2.4	1.4	0.3	0.969	0.129	0.876
Arachidonic (C20:4; n-6)	18.3	16.2	19.9	14.5	1.9	0.595	0.185	0.647
Eicosapentaenoic (EPA, C20:5; n-3)	0.2	0.1	0.2	0.1	0.1	0.833	0.833	0.188
Docosapentaenoic (DPA C22:5; n-3)	1.7	2.1	2.5	1.3	0.3	0.521	0.103	0.642
Docosahexaenoic (DHA, C22:6; n-3)	0.3	0.7	0.4	0.5	0.2	0.421	0.843	0.22
Other fatty acids	23.2	20.7	26.3	17.6	2.3	0.601	0.085	0.901
Saturated fatty acids	376.3	382	355.5b	402.8a	6.7	0.677	0.004	0.561
Monounsaturated fatty acids	445.0	458.3	470.6a	432.7b	7.1	0.364	0.02	0.258
Polyunsaturated fatty acids	144.0	133.8	147.5	130.4	7.7	0.518	0.288	0.328
Total lipids, g 100 g^{-1}	42.4	49.2	44.8	46.9	2.9	0.266	0.718	0.87

[a,b] Means of main factors with different superscript differ (P≤0.05).

*FAME's = fatty acid methyl esters; CP = crude protein; LPD = low-protein diet; CLA = conjugated linoleic acid; SEM = standard error of the mean.

Longissimus muscle

The reduction of dietary CP lowered linolelaidic acid concentration (Table 8) and increased arachidic acid (P≤0.05) in *longissimus* muscle (LM). The alpha-linolenic acid (C18:3; n-3) concentration was lower (P=0.08) in the pigs fed low-protein diets. The concentration of CLA isomers increased (P≤0.05) with the dietary CLA. The level of myristic, palmytic, stearic, palmitoleic, and linolelaidic acids increased (P≤0.05), while palmitolaidic, oleic, linoleic, eicosaenoic, and cis-11,14-eicosadienoic acids

were reduced with CLA supplementation to the diet. Total SFA increased and the MUFA decreased (P≤0.05) in the pigs fed diets supplemented with CLA. The interaction of CP×CLA changed (P≤0.05) the concentration of CLA isomers (c9,t11 and c11,t9), with higher concentration in the pigs fed diets with CLA; however, their concentration was lower in the pigs fed LPD.

Table 8 - Effect of crude protein and conjugated linoleic acid level on total lipids and fatty acids profile in *longissimus* muscle of pigs.

Fatty acids, % FAME´s*	CP		CLA concentration (%)			*P* Value		
	Control	LPD	0.0	1.0	SEM	CP	CLA	CP×CLA
Myristic (C14:0)	16.4	15.2	11.5b	20.1a	0.5	0.317	0.001	0.599
Cis 10-Pentaenoic (C15:1)	3.7	3.3	3.9	3.1	0.6	0.783	0.531	0.315
Palmitic (C16:0)	264.5	261.2	238.2b	287.6a	4.3	0.71	0.001	0.46
Palmitelaidic (C16:1)	1.7	1.6	2.3a	1.0b	0.2	0.885	0.002	0.29
Palmitoleic (C16:1)	37.5	34.4	25.9b	45.9a	1.6	0.367	0.001	0.715
Heptadecaenoic (C17:0)	2.5	2.1	2.1	2.5	0.2	0.361	0.283	0.933
Cis 10-heptadecaenoic (C17:1)	2.2	2.1	2.2	2.1	0.2	0.76	0.715	0.951
Stearic (C18:0)	123.5	130.2	120.2	133.5a	2.4	0.196	0.017	0.126
Elaidic (C18:1; n-9 *trans*)	2.0	1.3	1.2	2.1	0.5	0.526	0.442	0.771
Oleic (C18:1)	366.7	374.5	413.1a	328.1b	5.4	0.477	0.001	0.79
Cis-vaccenic (C18:1)	33.8	30.4	31.7	32.5	0.8	0.683	0.609	0.404
Linolelaidic (C18:2; n-6 *trans*)	1.3a	0.3b	0.6	1.0	0.1	0.001	0.051	0.287
Linoleic (C18:2; n-6)	88.4	80.6	92.5	76.4	4.9	0.435	0.124	0.115
c9,t11 y c11,t9 CLA	5.8	4.5	0.5b	9.8a	0.5	0.217	0.001	0.039
t10,c12 CLA	1.0	1.4	0.0b	2.5a	0.2	0.323	0.001	0.323
Other isomers of CLA	1.1	0.6	0.0	1.7	0.3	0.407	0.021	0.407
Gama-linolenic (C18:3)	0.2	0.6	0.2	0.7	0.2	0.278	0.164	0.919
Alfa-linolenic (C18:3; n-3)	4.4	3.7	5.1a	3.0b	0.2	0.084	0.001	0.168
Arachidic (C20:0)	2.0b	2.5b	2.3	2.2	0.1	0.002	0.844	0.186
Eicosaenoic (C20:1)	5.6	6.0	6.8a	2.8b	0.1	0.194	0.001	0.094
Cis-11, 14-eicosadienoic (C20:2)	3.4	3.1	3.7a	2.8b	0.2	0.335	0.011	0.143
Cis-11,14,17-eicosatrienoic (C20:3)	0.2	0.8	0.2	0.8	0.2	0.244	0.265	0.978
Cis-8,11,14-eicosatrienoic (C20:3)	1.4	1.0	1.6	0.9	0.3	0.472	0.224	0.584
Arachidonic (C20:4 ω6)	9.7	9.7	10.6	8.9	1.5	0.997	0.583	0.396
Eicosapentaenoic (EPA, C20:5; n-3)	0.0	0.1	0.0	0.1	0.7	0.337	0.337	0.337
Docosapentaenoic (DPA C22:5; n-3)	1.4	1.1	1.5	1.1	0.3	0.551	0.500	0.429
Docosahexaenoic (DHA, C22:6;n-3)	0.3	0.3	0.2	0.4	0.2	0.916	0.598	0.86
Other fatty acids	16.2	26	20.9	21.3	2.4	0.575	0.921	0.996
Saturated fatty acids	409.8	411.6	374.7b	446.7a	5.8	0.878	0.001	0.214
Monounsaturated fatty acids	453.8	454.0	487.5a	420.3b	5.0	0.982	0.001	0.815
Polyunsaturated fatty acids	120.2	108.2	116.9	111.5	7.2	0.423	0.714	0.243
Total lipids, g 100 g^{-1}	67.0	77.8	71.2	73.6	7	0.453	0.87	0.695

[a,b] Means of main factors with different superscript differ (P≤0.05). *FAME's = fatty acid methyl esters; CP = crude protein; LPD = low-protein diet; CLA = conjugated linoleic acid; SEM = standard error of the mean.

DISCUSSION

Growth performance

The reduction of average daily gain but not feed: gain ratio in the nursery pigs due to a higher reduction of CP as observed in the present study has also been earlier observed in the nursery pigs fed sorghum-soybean meal diet with 4% less protein (Hansen et al. 1993) and with corn-soybean meal diets with 4% (Kerr et al. 1995) or 5.0% (Le Bellego and Noblet 2002) less CP supplemented with crystalline amino acids. Reduction of CP by 1.5% during the growing phase did not affect the growth performance variables, but when CP was lowered from 16.0 to 11.5%, ADG and ADFI were diminished by 140 and 320 gd^{-1}, respectively. The reduction of ADG and ADFI could have been originated because a higher reduction would limit some AA such as isoleucine and valine, affecting negatively the response of the pigs (Figueroa et al. 2002). This lower response of the pigs fed low-protein diets could also be due to the reduction of nitrogen for the synthesis of non-essential AA (Tuitoek et al. 1997; Heger et al. 1998), because under adequate use of dietary protein, some of the essential AA

could be partly metabolized and used for the synthesis of non-essential AA (Heger et al. 1998). However, with the proper supply of essential and non-essential AA, the maximum growth performance (Kerr et al. 2003; Shriver et al. 2003; Deng et al. 2007) in the pigs fed low-protein diets could be obtained. The reduction of the negative impact on the production variables in low protein diets through the supplementation with synthetic amino acids in finishing diets has been reported with a dietary CP reduction up to 4% (Kerr et al. 1995; Knowles et al. 1998; Kerr et al. 2003). However, Figueroa et al. (2004) observed lower ADG and feed efficiency when dietary CP was reduced from 16 to 13% for 55-100 kg pigs (Tuitoek et al. 1997). In addition, FGR increased when CP was reduced (Kerr et al. 2003; Figueroa et al. 2004), which could be due to the limiting amounts of isoleucine and valine when dietary CP was reduced by 4.0% or more, becoming marginal or deficient (Liu et al. 1999).

CLA supplementation for the nursery and growing pigs did not improve the analyzed variables. It has been observed that the addition of 2% CLA increased the ADG by 5.0% and FGR by 7.0%, without affecting the ADFI (Bee 2001). However, in another study, the addition of 1% CLA from a source with 60.0% of CLA for 49-113 kg did not improve the growth performance (Averette-Gatlin et al. 2002) or with 1.0 or 2.0% of CLA for the gilts (Martin et al. 2008a), which was similar to results found in the present study.

The growing-finishing pigs fed diets with low fat concentration had better response to CLA addition (Dugan et al. 2001). However, fat concentration in the diets used in the present study was even lower than that by Dugan et al. (2001), and still there were no significant differences due to the inclusion of CLA. Schinckel et al. (2000) also used 1.0% of CLA in the diets for gilts with different genetic potential during fattening period which did not change the growth performance.

Carcass characteristics

Feeding the pigs with low-protein (LPD) AA-supplemented diets increased the body energy retention in the growing pigs, resulting in higher carcass fat (Tuitoek et al. 1997). This increment in energy retention as fat was observed when crude protein was reduced more than 3.0% in the diets for 100 kg pigs (Le Bellego et al. 2001). There are reports stating that the body fat increases in the pigs fed LPD supplemented with AA (Kerr et al. 1995; Gómez et al. 2002). In contrast, it has also been observed that the reduction of CP by 4% had similar effects on backfat thickness (Canh et al. 1998), or when CP was reduced from 16.6 to 13.0% (Tuitoek et al. 1997) in the growing or finishing pig diets. These results were similar to the present findings in the growing and finishing stages, but not in nursery pigs.

The FFLG and fat accumulation in the pigs did not change when CP was reduced by 4.0% (Kerr et al. 2003). However, in another study, FFLG and LMA were reduced in the same proportion as CP was lowered in the growing gilts without affecting backfat thickness (Figueroa et al. 2002). It also has been found that in spite of the reduction of LMA in the pigs fed low-protein diets, other carcass characteristics such as FFLG, backfat thickness, and lean meat percentage were not affected by the reduction of dietary protein (Kerr et al. 2003). Other studies reported no difference on LMA in the pigs fed standard or LPD supplemented with AA (Kerr et al. 1995; Knowles et al. 1998). The present results showed a lower LMA during the nursery and growing phases due to the CP concentration, as was found in other investigation (Liu et al. 1999).

The inclusion of 0.5% of CLA in growing-finishing diets produced no change in lean meat percentage (LMP) and backfat thickness (Lauridsen et al. 2005). In contrast, fat deposition was reduced by 31.0% with the addition of 1% CLA, and, consequently, the ratio fat: lean meat decreased with increasing amount of CLA. This low effect on the carcass traits in the pigs consuming CLA, found in this study has been reported by others with similar concentration (1.0%) and just an increase of 18.8% in marbling score in 49-113 kg pigs (Averette-Gatlin et al. 2002). In addition, the lean meat deposition had a quadratic response to the dietary CLA concentration, finding the maximum response at 0.5% CLA for the finishing pigs (Ostrowska et al. 1999). The LMA did not change by CLA concentration up to 2%; backfat thickness increased in the growing pigs fed 0.25 and 0.5% CLA compared with corn oil (Ramsay et al. 2001). Hence, during this stage, adding CLA had no benefit and probably the positive effect on lipid accumulation was more noticeable in the finishing pigs. Furthermore, the addition of CLA in the diets would be acceptable if the cost of CLA in the diet could be covered by value-added carcass and better meat quality (better marbling, less fat, more

firmness; Wiegand et al. 2001).The inclusion of 2.0% CLA did not affect the LMP and LMA in 70-106 kg pigs. The backfat thickness was lower in the pigs fed CLA than those fed linoleic acid or lard (Bee 2001). In another study, supplementation of 1 or 2% CLA did not affect the carcass characteristics of finishing pigs fed *ad libitum* (Martin et al. 2008a), or with 2% in the pigs restricted-fed (Migdal et al. 2004). The carcass of pigs fed up to 1.0% of CLA isomers during the growing-finishing phase showed lower subcutaneous fat; the LMA was reduced with CLA concentration higher than 0.5% with direct measurement, but it increased by the addition of the CLA when measured by ultrasound (Thiel-Cooper et al. 2001).

In barrows, the addition of CLA isomers (1.5 or 3.0 g kg^{-1}) during the period of rapid accumulation of fat (90-120 kg) reduced fat deposition and increased LMP (Su et al. 2006). Hence, the period of CLA feeding was an important factor in the response of the pigs (Azain 2003). In TBP pigs (TLRI Black Pig; a type of fat pig), fat content in the meat increased when the period of feeding the CLA was longer (Su et al. 2006). Wiegand et al. (2002) reported that the inclusion of 0.75% of CLA isomers in 56-115 kg pigs increased the LMA and reduced backfat thickness; this effect was the same if the feeding period started at 28 kg. In 63.8-98.9 kg barrows with longer CLA intake time (3 *vs.* 6 weeks) in high concentration (4.0%), backfat decreased and LMA and intramuscular fat increased (Sun et al. 2004). In the present study, the feeding period was longer than the above study without effect on the mentioned variables. This suggested that fatter pigs responded better to CLA treatment than the pigs with higher genetic potential for lean gain and lower backfat thickness (Azain 2003). Despite this, CLA improved the carcass quality in genetically lean meat pigs by changing the fatty acid concentration and reducing backfat thickness, an effect that was a function of feeding time of CLA supplemented diet (Schinckel et al. 2000).

Plasma urea nitrogen concentration

Reducing dietary crude protein reduced the linearly PUN concentration (Figueroa et al. 2002), as was observed in this study during growing-finishing phase. A lower PUN concentration was also related to a lower metabolic heat production associated to the synthesis and excretion of urea from the dietary AA excess (Kerr et al. 2003). The reduction of PUN in the pigs fed ideal protein diets compared with standard intact protein indicated that there was AA excess in the standard diets. In addition, pigs fed low-protein diets used more efficiently dietary protein than the pigs fed standard diets; those pigs retained similar amount of nitrogen than the pigs fed a standard diet if the reduction of crude protein was adequate (Shriver et al. 2003). The fecal and urinary nitrogen excretion could be reduced by 10% for each percentage unit that crude protein was reduced (Shriver et al. 2003). The urinary nitrogen excretion of pigs is linearly and positively related to PUN concentration. Hence, reducing the crude protein is an alternative to lower total nitrogen excretion and an indirect way to measure the amount of nitrogen retention and waste (Zervas and Zijlstra 2002). Feeding LPD indirectly reduces the ammonia production due to the dietary nitrogen reduction (Canh et al. 1998; Hayes et al. 2004), but if the reduction of crude protein is not adequate, growth performance and carcass characteristics could be adversely affected.

The dietary 1.0% CLA supplementation for growing pigs (Ramsay et al. 2001) or 0.75% for finishing (105-153 kg) pigs (Corino et al. 2008) did not affect the PUN concentration, as also was observed in the present study, indicating no effect on protein metabolism and that its function was directed to lipid metabolism.

Fatty acid profile in meat

The accumulation of CLA isomers in intramuscular fat of *semimembranosus* (SM) and *longissimus* muscle (LM), found had been previously reported mainly for LM (Migdal et al. 2004) and its concentration was related to the CLA level included in the diet (Ramsay et al. 2001; Joo et al. 2002). The c9,t11 and c11,t9 are the main isomers accumulated in the muscle of pigs (Thiel-Cooper et al. 2001; Lauridsen et al. 2005; Martin et al. 2008b).

The change in the fatty acid composition due to CLA intake increases the proportion of saturated to unsaturated fatty acids in intramuscular fat. This can improve the water holding capacity in the meat due to lower content of linoleic acid (Joo et al. 2002). However, the reduction of this fatty acid could adversely affect the nutritional quality of the meat (Teye et al. 2006).

CONCLUSIONS

Reducing the crude protein concentration in the diet of fattening pigs from 20.5 to 16.0% in the nursery phase, from 16.0 to 14.5% in the growing stage, and from 14.0 to 12.5% in the finishing pigs did not negatively affect the growth performance, nor carcass characteristics. The addition of conjugated linoleic acid to low-protein or standard diets did not improve the growth performance or carcass characteristics, although it improved the concentration of some fatty acids in the meat. Hence, its addition to diet would depend on the advantage of its cost compared to other energy sources such as soybean oil.

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