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Effect of dietary conjugated linoleic acid on chemical and fatty acid composition of pig skeletal muscle and subcutaneous adipose tissue

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A b s t r a c t: Relationships among conjugated linoleic acid (CLA) in pig nutrition, chemical composition of meat and fatty acids composition of meat and adipose tissue were determined in pigs (crossbreed Yorkshire x Landrace) (n=60) A commercial CLA preparation containing 60% of CLA isomers was added to the pig diet. SFA and MUFA in the control pig diet (with no added CLA) were significantly higher (p<0.01) than SFA and MUFA in the experimental pig diet. CLA supplementation in the pig diet significantly increased SFA and PUFA and decreased MUFA in both meat and adipose tissue. The same content of c9t11CLA and t10c12CLA was found in the supplemented pig diet. Both c9t11CLA and t10c12CLA were found in meat and adipose tissue of pigs consuming the experimental diet, but not in meat and adipose tissue of pigs consuming the control diet.

Keywords: *pig nutrition, chemical composition, fatty acids, meat, CLA.*

Introduction

The production of high quality pork has been a constant objective of the pig industry for many decades. The main goal is to obtain pigs with high lean percentage and good meat quality traits at the same time (*Baltic et al.*, 2011; *Dokmanovic et al.*, 2015; *Dokmanovic et al.*, 2016). Fat and fatty acids (FAs), whether in adipose tissue or muscle, importantly contribute to the various aspects of meat quality. Several nutritional attempts to modify fat and FAs in pigs have been attempted recently; one of them is the addition of conjugated linoleic acid (CLA) in the diet for growing/finishing pigs to increase CLA in muscle and adipose tissue (*Ivanovic et al.*, 2015; *Markovic et al.*, 2015). CLA is a group of positional and geometric isomers of linoleic acid (C18:2), which were first identified in rumen fluid as an intermediate of the biohydrogenation process. In synthetic CLA preparations, the c9,t11 and t10,c12 isomers are predominant (often in a 1:1 ratio). It appears that the c9t11 isomer has positive effects on some types of cancer by inhibiting tumourogenesis, while t10c12 isomer could be responsible for changes in fat deposition (*Pariza et al.*, 2000). In addition, dietary CLA seems to be highly deposited in body tissues of monogastric animals and as a result, in pork and meat products (*Bee*, 2001; *Corino et al.*, 2005).

Most reports in which the effect of CLA on FAs composition was evaluated were performed in muscle. Therefore, the aim of this study was to evaluate effect of CLA on chemical composition of meat and FA composition of meat and adipose tissue.

Materials and Methods

Animals and diets

Crossbreed Yorkshire x Landrace pigs, with initial body weight of 60 kg were divided into two groups of 30 pigs each and fed a standard mixture (*National Research Council*, 1998), formulated to meet maintenance and growth requirements of animals during their growth from 60 to 110 kg (fattening period of 60 days). Commercially prepared CLA (60%) (Lutalin™, BASF, Germany), was added to the feed of the experimental pigs at the recommended rate of 2% in the feed mixture. The nutrient composition of the diets is shown in Table 1.

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Table 1. Ingredients and chemical composition of the pig diets (%)

Ingredients	Control diet	Experimental diet
Corn	48	46
Barley	28	28
Soybean meal	16	16
Wheat bran	5	5
Lutalin	–	2
Di-Ca-P	0.60	0.60
Cattle chalk	1.20	1.20
Cattle salt	0.40	0.40
Premix	1.16	1.16
Chemical composition		
Moisture	12.28	12.22
Proteins	15.24	15.39
Fat	2.968	2.984
Crude fibre	4.088	4.206
Crude ash	2.64	2.78
Ca	0.77	0.77
P	0.52	0.52
ME-s	12.66	12.66
Lys	0.89	0.89
Met+Cyst	0.54	0.54

Legend: Control diet without addition of CLA); Experimental diet with addition of CLA); Premix composition (per kg): Vit. A 700000IJ; Vit. D3 100000IJ; Vit. E 1200 mg; Vit. K3 100 mg; B1 200mg; B2 250 mg; B6 150mg; B12 1.5 mg; Biotin 5 mg; Ca-pantothenate 1200 mg; Niacine 1500 mg; Choline chloride 50000 mg; Fe 10000mg; Cu 2000 mg; Mn 2500 mg; Zn 10000mg; J 90 mg; Se 10 mg; Co50 mg; Helmox (antioxidant) 10000 mg

Chemical methods

Complete mixtures of the two pig diets were sampled to determine the FA composition at the beginning of the study. Meat (*Longissimus thoracis et lumborum*) and fat samples were taken from ten pigs in experimental and control groups, after slaughtering, processing and chilling of carcasses, for chemical analysis and analysis of FA composition. Chemical analyses to determine protein, water, fat and mineral content were conducted according to AOAC methods (*AOAC*, 1990). Lipids from subcutaneous back fat were extracted by the procedure proposed by Bligh and Dyer (1959). Total lipids for FA determination were extracted from pig muscle tissue with a hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Germany). After evaporation of solvent until dryness under the stream of nitrogen, total lipids were converted to FA methyl esters (FAMEs) by trimethylsulphonium hydroxide. FAMEs were determined by using Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cyanopropyl HP-88 capillary column (100m x 0.25 mm x 0.20μm) (*Trbovic et al.*, 2011). Temperature of the injector and detector were 250°C and 280°C, respectively. The carrier gas was nitrogen at a flow rate of 1.33 mL min^{-1} and split ratio of 1:50. Injection volume was 1 μL. Column oven temperature was programmed in the range from 125°C to 230°C. The total run time was 50.5 min. FAMEs were identified on the basis of the relative retention time compared with the relative retention times of the individual compounds in the standard FAME mixture Supelco Component 37 FAME mix (Supelco, Bellefonte, USA). Quantification of FAs was determined relative to an internal standard, heneicosanoic acid, C21:0. The content of FAs is expressed as a percentage (%) of the total identified FAs.

Statistical Analysis

Statistical analysis of the results was conducted using software GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego CA, USA, www.graphpad.com. All parameters were described by descriptive statistics (mean±standard deviation). Student's t-test was used to determine the significance of differences between the control and experimental group. Values of $p<0.01$ and $p<0.05$ were considered significant.

Results and Discussion

Fatty acid composition of the animal diets

Significant differences between the FA composition of the diets for control and experimental pigs were found. SFA and MUFA were significantly higher ($p<0.01$) in the control pig diet compared to the experimental diet. Polyunsaturated FAs (PUFA), n-3 and n-6 were significantly higher ($p<0.01$) in the experimental diet. A more beneficial n-6/n-3 ratio was seen in the control pig diet ($p<0.05$) (Table 2).

The CLA isomers c9t11CLA and t10c12CLA were detected in the diet with CLA (the experimental diet). The content of c9t11CLA was 2.57±0.02%, while that of t10c12CLA was 2.55±0.01 (Table 3). CLA, in nature, originates mainly from bacterial isomerisation or/and biohydrogenation of PUFA in the rumen and from desaturation of trans FAs in the adipose tissue and mammary gland. In the rumen, the bacterial microbiota produces the enzymes linoleate isomerase and CLA-reductase which convert unsaturated FAs in fat metabolism to CLA or important intermediate CLA precursors, enabling the end product, stearic acid. The endogenous synthesis from trans-vaccenic acid was also

Table 2. Fatty acid composition in feed (% of total fatty acids)

Fatty acid, %; ratio	Control diet ($\overline{X}$±SD)	Experimental diet ($\overline{X}$±SD)
SFA	22.39^{A}±0.40	17.93^{A}±0.27
MUFA	38.31^{A}±0.20	25.71^{A}±0.24
PUFA	39.00^{A}±0.60	56.36^{A}±0.32
n-3	2.37^{A}±0.07	3.37^{A}±0.22
n-6	36.87^{A}±0.55	47.84^{A}±0.18
n-6/n-3	15.54^{a}±0.31	20.00^{a}±0.21
C14:0	0.13^{A}±0,01	0.09^{A}±0.004
C15:0	0.04±0,004	0.04±0.008
C16:0	16.44^{A}±0,27	14.57^{A}±0.23
C17:0	–	0.22±0.03
C18:0	4.48^{A}±0.08	2.21^{A}±0.03
C20:0	0.46±0.04	0.45±0.04
C22:0	0.58^{A}±0.007	0.21^{A}±0.009
C24:0	0.25^{A}±0.008	0.21^{A}±0.005
C16:1	0.09^{A}±0.009	0.11^{A}±0.005
C18:1	38.22^{A}±0.21	25.43^{A}±0.24
C18:2 n-6	36.60^{A}±0.16	47.27^{A}±0.58
C20:2 n-6	0.02±0.002	0.02±0.003
C20:3 n-3	1.47^{A}±0.22	2.37^{A}±0.07
C18:3 n-6	0.21^{A}±0.009	0.58^{A}±0.007
c9t11CLA	**ND**	**2.57±0.02**
t10c12CLA	**ND**	**2.55±0.01**
c9t11CLA+ t10c12CLA	**ND**	**5.12±0.03**

Legend: $^{A, B, C}$Same letters indicate significant difference of $p<0.01$; ^{a}Same letters indicate significant difference of $p<0.05$; ND not detected

Table 3. Chemical composition of meat from pigs fed different diets (mean±standard deviation)

Parameters (%)	Pigs fed control diet[A]	Pigs fed experimental diet[B]
Moisture	69.00±0.14	69.49±0.01
Proteins	21.74±0.03	21.66±0.09
Fat	8.32[a]±0.08	7.90[a]±0.05
Ash	0.94±0.01	0.95±0.01

Legend: [A]Control diet without addition of CLA; [B] Experimental diet with added CLA; [a] same letters indicate significant difference of $p<0.05$

documented in humans but the predominant source of CLA seems to be the dietary CLA intake via meat and meat products as well as milk and dietary products. The commercial CLA preparation used in the current study contains equal amounts of c9t11CLA and t10c12CLA isomers (*Eggert et al.*, 2001). The efficiency of CLA enrichment on products of animal origin (meat, milk, eggs) varies primarily depending on the species and concentration of CLA in diet (*Wiegand et al*., 2001; *Joo et al.*, 2002; *Wachira et al.*, 2002; *Kott et al.*, 2003; *Lauridsen et al.*, 2005; *Bee et al.*, 2008; *Cordero et al.*, 2010; *Markovic et al.,* 2013; *Tous et al.*, 2013).

Chemical composition of meat

The chemical composition of the meat (protein, fat, water and ash) is shown in Table 2. The moisture content in meat from control animals (consuming the control diet) was 69.00±0.14% and in meat from experimental pigs (consuming the experimental diet) was 69.49±0.01%. Meat from control animals contained 21.74±0.03% protein, while meat from experimental animals contained 21.66±0.09% protein. The fat content in meat from control and experimental pigs was 8.32±0.08% and 7.90±0.05%, respectively, with a significant difference between groups ($p<0.05$) (Table 3). The ash content in meat from the control and experimental groups was approximately the same, being 0.94±0.01% and 0.95±0.01%, respectively. These results were similar to those of other studies (*Lawrie,* 1991; *Dokmanovic et al.,* 2015; *Djordjevic et al.,* 2016).

Fatty acid composition of meat and adipose tissue

The content of SFA and PUFA in the meat and adipose tissue of control pigs (those consuming a control diet) was significantly higher ($p<0.01$) than in experimental pigs. The MUFA content was significantly lower ($p>0.05$) in the meat and adipose tissue of control pigs. There were no significant differences in the contents of n-3 and n-6 FAs between the meat of control and experimental pigs. The significant differences between SFA, MUFA and PUFA are shown in Tables 4 and 5.

The meat of pigs fed the experimental diet contained 2.37±0.01% of c9t11CLA and 1.19±0.01% of t10c12CLA (Table 4), while adipose tissue contained 2.86±0.07% of c9t11CLA and 83±0.01% of t10c12CLA1 (Table 5). CLA was not detected in the meat or adipose tissue of pigs consuming control pigs.

Previous studies have observed an increase of SFA and a reduction of MUFA in subcutaneous backfat due to dietary CLA (*Bee*, 2001; *Demaree et al*. 2002; *Smith et al*., 2002). In the first studies with pigs, dietary CLA increased lean tissue deposition and decreased fat deposition (*Ostrowska et al.*, 1999). Comprehensive reviews on the effects of CLA on growth performance and carcass fat deposition in pigs have been published by *Corino et al.* (2005) and *Bee et al.* (2008). In general, the effects of adding CLA to pig diets were inconclusive. Inconsistency could be attributed to the pig breeds used in different studies or sources of CLA, the dietary fat content or feeding duration (*Tous et al*., 2013).

A higher amount of CLA was used in order to increase the level of CLA in meat. *Bee* (2001) reported that CLA-enriched oil (2%) supplemented in the diet of Swiss Large White pigs weighting from 70 to 105 kg resulted in a measurable CLA content (14.9 mg g^{-1} of FAs) in the adipose tissue, compared with non-detectable CLA levels in the pigs with linoleic acid-enriched oil or lard supplements.

Several reports indicate that CLA supplementation increases the amount of saturated FAs (C14:0, C16:0, and C18:0) and decreases the MUFA fraction (mainly C18:1) in pig tissues by down-regulating the D9-desaturase activity (*Bee*, 2001; *Eggert et al*., 2001; *Thiel-Cooper et al.,* 2001; *Smith et al*., 2002; *Lauridsen et al*., 2005).

It is known that the CLA can contain at least 28 different isomers, but only the two major isomers

(9c,11t CLA and 10t,12c CLA) have been evaluated in most studies performed in pigs, although the rest could also have some important roles in metabolism (*Tous et al.*, 2013). The CLA supplements used in the studies below generally consisted of a mixture of a limited number of CLA isomers (c9t11CLA and t10c12CLA). Glaser *et al*. (2002) analysed muscle tissue (*m. Longissimus dorsi*) of Large White pigs fed a barley–wheat–soybean meal-based diet with 6% of high-oleic sunflower oil or different amounts (1.85%, 3.70%, 5.55%) of partially hydrogenated rapeseed oil (high in trans FAs), from 30 to 103 kg. They reported increased amounts of CLA in muscle tissue of pigs fed the diet with added partially hydrogenated rapeseed oil (3.8, 6.4, and 8.5 mg CLA g^{-1} of FAME) and 0.9 mg g^{-1} of FAME in the sun-flower oil control pigs.

Cordero *et al.* (2010) found increased SFA (mainly C16:0) in pig meat after ingestion of dietary CLA. This shift towards greater saturation in all tissues and a decrease in MUFA in muscle could lead to reduced lipid oxidation of the adipose tissue and improve the meat technological properties, but could have a hypercholesterolaemic effect for the consumer. These changes in saturation reflect a reduction of Δ-9 desaturase activity by CLA (*Smith et al*., 2002). However, oleic acid was more reduced by CLA than palmitoleic acid, indicating that the inhibition of Δ-9 desaturase may be less pronounced for palmitic acid (*Smith et al*., 2002).

Table 4. Fatty acid composition in muscle derived from pigs consuming a control or experimental diet (% of total fatty acids)

Fatty acid, %; ratio	**Control diet** ($\overline{X}$±SD)	**Experimental diet** ($\overline{X}$±SD)
SFA	43.08^{A}±1.38	49.51^{A}±1.07
MUFA	46.57^{A}±1.88	37.35^{A}±0.28
PUFA	9.95^{a}±0.60	12.79^{a}±0.92
n-3	0.47±0.04	0.48±0.03
n-6	9.36±0.57	8.39±0.90
n-6/n-3	19.91^{a}±2.60	17.48^{a}±1.97
C14:0	1.08^{A}±0.01	2.01^{A}±0.07
C15:0	0.04±0.008	0.05±0.01
C16:0	27.22^{A}±0.24	29.98^{A}±1.16
C17:0	0.33±0.05	0.32±0.03
C18:0	14.72^{A}±1.08	17.45^{A}±0.16
C20:0	0.24^{a}±0.03	0.20^{a}±0.01
C16:1	2.38^{A}±0.24	3.40^{A}±0.36
C18:1	43.26^{A}±1.70	33.95^{A}±0.59
C20:1	0.93±0.08	ND
C22:1+C20:4	0.33±0.04	0.36±0.01
C18:2 n-6	8.83±0.54	7.91±0.85
C18:3 n-3	0.35^{A}±0.02	0.30^{A}±0.02
C20:2 n-3	0.42±0.06	0.39±0.04
C20:3 n-6	0.11^{A}±0.01	$0,09^{A}$±0.01
C20:3 n-6	0.12^{A}±0.01	$0,18^{A}$±0.01
c9t11CLA	**ND**	**2.37±0.01**
t10c12CLA	**ND**	**1.19±0.01**
c9t11CLA+ t10c12CLA	**ND**	**3.56±0.71**

Legend: ^{A}Same letters indicate significant difference of $p < 0.01$; ^{a}Same letters indicate significant difference of $p < 0.05$; ND not detected

Table 5. Fatty acid composition in adipose tissue derived from pigs consuming a control or experimental diet (% of total fatty acids)

Fatty acid, %; ratio	Control diet ($\overline{X}$±SD)	Experimental diet ($\overline{X}$±SD)
SFA	41.68^{A}±0.38	50.54^{A}±0.96
MUFA	44.62^{A}±0.99	31.28^{A}±0.35
PUFA	13.70^{A}±0.78	18.18^{A}±0.71
n-3	0.50±0.06	0.54±0.03
n-6	13.11±0.71	12.71±0.68
n-6/n-3	18.69^{a}±1.55	17.37^{a}±0.54
C14:0	1.03^{A}±0.04	2.24^{A}±0.16
C15:0	0.06^{A}±0.01	0.09^{A}±0.01
C16:0	25.63^{A}±0.48	29.64^{A}±0.98
C17:0	0.43^{a}±0.07	0.54^{a}±0.08
C18:0	14.26^{A}±0.40	17.82^{A}±0.25
C20:0	0.28±0.02	0.27±0.02
C16:1	2.02^{A}±0.09	1.60^{A}±0.10
C18:1	41.42^{A}±0.93	29.53^{A}±0.36
C20:1	0.99±0.04	ND
C22:1+C20:4	0.29^{A}±0.02	0.16^{A}±0.04
C18:2 n-6	12.15±0,71	11.79±0.64
C18:3 n-3	0.53^{A}±0,03	0.58^{A}±0.02
C20:2 n-6	0.59±0,01	0.61±0.03
C20:3 n-6	0.16^{A}±0,01	0.11^{A}±0.01
C20:3 n-3	0.16^{A}±0,001	0.14^{A}±0.001
c9t11CLA	**ND**	**2.86±0.07**
t10c12CLA	**ND**	**1.83±0.01**
c9t11CLA+ t10c12CLA	**ND**	**4.69±0.65**

Legend: ^{A}Same letters indicate significant difference of $p<0.01$; ^{a}Same letters indicate significant difference of $p<0.05$; ND not detected

Feeding gilts a conventional corn–soybean meal diet supplemented with either 1% CLA oil or 1% sunflower oil from 75 to 120 kg live weight resulted in a higher CLA concentration in the *m. Longissimus dorsi* of gilts fed a diet with added CLA oil (5.5 vs. 0.9 mg g^{-1} of FAs) (*Eggert et al.,* 2001). Similar results (4.4 vs. 0.8 mg g^{-1} of FAME in *m. Longissimus dorsi*) were recently published by *Lauridsen et al*. (2005) who supplemented the diet of 100 Danish barrows either with 0.5% CLA or 0.5% sunflower oil from 40 to 100–130 kg live weight.

Demaree et al. (2002) fed pigs from the 17th day of age with corn-soybean meal diets supplemented with either tallow or corn oil with or without 3% CLA for 35 days and found CLA content of 27.3 and 23.0 mg g^{-1} of FAs in *m. Longissimus dorsi* (corn oil with CLA and tallow with CLA, respectively). The CLA content in meat could be further enhanced if CLA supplementation is combined with additional dietary fat (*Markovic et al.*, 2015).

Conclusion

Addition of the recommended amount of dietary CLA for conventional genotype pigs (Yorkshire x Landrace) increased the CLA in meat and adipose tissue. Also, the addition of CLA to the pig diet increased the SFA and PUFA contents and reduced the MUFA content in both meat and adipose tissue. The results from this study showed that pig products can be enriched with CLA to provide a significant increase in the level of functional lipids, which could have positive influences on human health.

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