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Effect of linseed and the combination of conjugated linoleic acid and linseed on the quality and oxidative stability of pig meat and subcutaneous fat

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ABSTRACT: The aim of this experiment was to test the hypothesis that conjugated linoleic acid (CLA) in diets of finishing pigs fed linseed can improve the quality and oxidative stability of meat and subcutaneous fat. Twenty-four Prestice Black-Pied pigs (barrows and gilts) were divided into three groups and were fed a basal diet and diets supplemented with ground linseed (70 g/kg), or linseed combined with conjugated linoleic acid (20 g CLA-oil/kg). The trial duration was 53 days. Measurements included slaughter and meat quality parameters, oxidative stability determination, and fatty acid profile of meat and subcutaneous fat. The experimental data were analysed using one-way analysis of variance. Neither linseed nor linseed with CLA significantly influenced weight gain, lean percentage, muscle depth, backfat thickness, drip loss, meat shear force, dry matter, intramuscular fat or cholesterol ($P > 0.05$). Dietary supplementation with linseed increased the percentage of linolenic acid in the fatty acids of meat and backfat and resulted in higher production of aldehydes. Dietary CLA did not influence the susceptibility of lipids to oxidation. Supplementation with CLA significantly increased CLA proportions in fatty acids of meat and backfat, reduced proportions of monounsaturated fatty acids, and increased proportions of saturated fatty acids in backfat ($P < 0.05$). The concentration of CLA (in mg/100 g of fresh tissue) in backfat was almost fifty times higher than in meat. Both meat and backfat of pigs fed CLA-free diets contained CLA, probably as a result of microbial conversion of linoleic acid in the intestine. It can be concluded that CLA changed the fatty acid profile of meat and backfat, but did not improve oxidative stability and other meat quality traits of pigs fed linseed.

Keywords: CLA; meat quality; backfat; TBARS; fatty acids

Linseed is rich in unsaturated fatty acids (UFA), primarily α-linolenic acid, followed by oleic and linoleic acids. Teneva et al. (2014) showed that in triacylglycerols of four genotypes of linseed linolenic acid represented 33.5–45.8% of fatty acids (FA). Feeding linseed to pigs increases the content of n-3 polyunsaturated fatty acids (PUFA) in muscle and adipose tissue (α-linolenic, eicosapentaenoic and docosahexaenoic acids), thus improving the nutritional quality of pork (Corino et al. 2014). However, the increased concentration of UFA worsens the technological quality of pork in the meat processing industry because of inferior muscle and adipose tissue cohesiveness on cutting, lower fat firmness, and higher susceptibility to rancidity development (Nishioka and Irie 2006). Undesirable effects of UFA limit the use of plant oils and oilseeds in pig diets. Warnants et al. (1995) suggested that diets of fattening pigs can contain up to 18 g PUFA/kg of feed and increasing the PUFA content above this level resulted in weaker backfat.

Fat softness was associated with decreased percentages of palmitic, stearic and oleic acids, and increased proportions of linoleic and linolenic acid (Maw et al. 2003). Thus, supplements that have the potential to modify the FA profile have attracted attention. Feeding conjugated linoleic acid (CLA) represents a feasible strategy to modify the FA

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profile of meat and backfat in pigs fed oilseeds. There are numerous reports on the use of CLA-oil in pigs, which were at first focused on the fat-to-lean repartitioning and feed conversion (Dugan et al. 1997), carcass composition (Thiel-Cooper et al. 2001), and pork quality (Dugan et al. 1999). Pigs fed CLA deposited less subcutaneous fat (Dugan et al. 1997; Wiegand et al. 2001), and gained more lean than pigs fed a control diet (Thiel-Cooper et al. 2001). Independent of the tissue, pigs fed CLA exhibited higher levels of saturated fatty acids (SFA), and lower levels of monounsaturated fatty acids (MUFA) than pigs fed lard (Bee et al. 2001). The shift towards higher deposition of SFA and lower deposition of MUFA was observed also in lipids of other animal species: broiler chicks (Simon et al. 2000), rabbits (Marounek et al. 2007), and calves (Marounek et al. 2008). Modification of the FA profile has attracted attention as meat containing less UFA and more SFA may be less prone to lipid oxidation. The increase in the SFA/UFA ratio could have negative health implications (Watts et al. 1996; Williams 2000); thus, the inclusion of high levels of MUFA in the pig diet could counteract the decrease in MUFA (Martin et al. 2008c).

The effects of dietary CLA on fat deposition and oxidative stability of meat are not easy to predict. Thus, the aim of this work was to study the effect of linseed and the combination of CLA and linseed in pig diets on meat quality, and oxidative stability of meat and subcutaneous fat. Meat of pigs fed linseed can contribute substantially to long-chain n-3 FA intake in humans, particularly for societies in land-locked countries with low seafood consumption (Turner et al. 2014).

MATERIAL AND METHODS

Animals and diets. Twenty-four Prestice Black-Pied pigs (12 barrows and 12 gilts) weighing on average 71.9 ± 8.5 kg were divided into three groups. Pigs were group-housed in three pens, 4 × 4 m, eight pigs per pen. Individual pigs were experimental units. The ingredients and chemical composition of the diets are shown in Table 1. Pigs in the control group were fed the basal diet, pigs of experimental groups were fed diets supplemented with ground linseed (70 g/kg), or linseed combined with CLA-oil (20 g/kg). Pigs had access to their diets on an *ad libitum* basis. Commercial CLA-oil Lutalin® (BASF, Germany) is an oil containing 60% of CLA methyl esters. Linseed and CLA-oil were supplemented at the expense of rapeseed meal and rapeseed oil, respectively. After 53 days the pigs were weighed and their feed was removed 12 h before slaughter.

Slaughter measurements. Data on the lean percentage, muscle depth and backfat thickness were obtained from the slaughterhouse, where carcasses were classified by the ZP method of the SEUROP system (EU decision 2005/1/ES). Samples of meat and backfat were collected 24 h after slaughter, between the second and third last rib and transported in a portable fridge to the laboratory. The muscle depth and backfat thickness were measured between the second and third last rib. Estimation of the drip loss was performed during the period of 24–48 h after slaughter, by weighing 150 g of meat hanging in a bag at 5 °C.

Analyses. The dry matter of the meat was determined by oven drying at 105 °C and firmness using a Warner-Bratzler shear machine (Instron 3360, Canton, MA, USA). Samples were then packed in PE bags and stored at –20 °C until analysis. The oxidative stability of meat and backfat was measured using the thiobarbituric acid method of Piette and Raymond (1999) and results were expressed as thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde per kg meat. The content of intramuscular fat was determined according to ISO 1444 (1997) by extraction in a Soxtec 1043 apparatus (FOSS Tecator AB, Hoganas, Sweden). Fatty acid compositions of meat and backfat were determined after chloroform-methanol extraction of total lipids (Folch et al. 1957). Alkaline trans-methylation of fatty acids was performed as described by Raes et al. (2003). Gas chromatography of fatty acid methyl esters (FAMEs) was carried out using an Agilent 6890M chromatograph (Agilent Technologies, Inc., local distributor HPST Ltd., Prague, Czech Republic) equipped with a 60 m DB-23 capillary column (150–230 °C). Fatty acids were identified on the basis of retention times corresponding to standards. PUFA 1, PUFA 2, PUFA 3 and 37 Component FAME Mix standards (Supelco, Bellefonte, USA) were used. Standards of conjugated methyl esters of CLA *cis*-9, *trans*-11 and CLA *trans*-10, *cis*-12 were obtained from Sigma-Aldrich, Ltd. (Prague, Czech Republic). CLA isomers in Lutalin® and tissues were determined using a Shimadzu VP series instrument equipped with three Ag-impregnated

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Table 1. Ingredients and chemical composition of diets

	Diet		
	control	linseed	linseed + CLA
Ingredients (g/kg)			
Wheat	624	624	624
Barley	120	120	120
Soyabean meal, extracted	80	80	80
Rapeseed meal	70	–	–
Wheat bran	30	30	30
Linseed	–	70	70
Malt sprouts	30	30	30
Rapeseed oil	25	25	5
CLA-oil	–	–	20
Limestone	14.5	14.5	14.5
Salt	4	4	4
Monocalcium phosphate	4.5	4.5	4.5
Magnesium oxide	1	1	1
Sodium bicarbonate	1	1	1
Amino acids and vitamins	9	9	9
Nutrients (g/kg)			
Starch	440	409	401
Crude protein	162	154	151
Fat	48.0	62.5	82.5
Crude fibre	37.7	49.7	48.7

ChromSpher 5 Lipids 250 × 4.6 mm columns (Agilent Technologies, Inc.), in conjunction with a guard column (10 × 3 mm), containing the same stationary phase. Heptane with 1.2 ml/l acetonitrile was the mobile phase. The isocratic system was operated at 27 °C on a column with a flow rate of 0.9 ml/min. Isomers *trans*-10, *cis*-12 and *cis*-9, *trans*-11 were identified on the basis of retention times (the former isomer was eluted first). Other CLA isomers were identified on the basis of comparison of the UV spectra of methylesters of CLA (Czauderna et al. 2003). Peaks of absorbance at 231.9, 234.3 and 235.4 nm are characteristic of isomers *trans*-, *trans*-CLA, *cis*-, *trans*-/*trans*-, *cis*-CLA and *cis*-, *cis*-CLA, respectively.

To determine cholesterol, lipids were saponified and unsaponified matter extracted with diethyl ether according to ISO 3596 (1988). Silyl derivatives were prepared using TMCS and HMDS silylation reagents (Sigma-Aldrich), and quantified on a gas chromatograph equipped with a SAC-5 capillary column (Supelco), operated at 285 °C.

Treatment effects were evaluated by a one-way analysis of variance using the GLM procedure of SAS, version 8.2 (SAS Institute, Cary, USA). Differences ($P < 0.05$) were identified using Tukey's test.

RESULTS

Weight gains in pigs fed the control diet, diet supplemented with linseed, and diet with CLA and linseed were 759 ± 111, 722 ± 87 and 726 ± 124 g/day, respectively ($P > 0.05$). Neither linseed nor linseed with CLA influenced ($P > 0.05$) lean percentage, muscle depth, drip loss, shear force, meat dry matter, intramuscular fat or cholesterol (Table 2). Backfat thickness in pigs fed the control diet, diet supplemented with linseed, and diet with CLA and linseed was 23.4 ± 2.8, 21.1 ± 2.4 and 22.1 ± 5.2 mm, respectively ($P > 0.05$). Production of aldehydes was increased during the storage of meat and backfat samples (Table 3). Linseed supplementation increased values of TBARS in samples of meat stored for one or three days ($P < 0.05$), but not in samples of backfat ($P > 0.05$). Compared to the control diet, linseed combined with CLA increased TBARS in samples of backfat stored for one and six days ($P < 0.05$).

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Table 2. Meat quality of pigs fed the control diet or diets containing linseeds or CLA-oil

	Diet			SEM	*P*
	control	linseed	linseed + CLA		
Lean percentage (%)	51.1	53.1	52.2	0.7	0.426
Muscle depth (mm)	62.0	66.6	65.7	1.0	0.085
Backfat thickness (mm)	23.4	21.1	22.1	0.8	0.468
Drip loss (%)	2.37	2.51	2.32	0.07	0.483
Shear force (N)	72	66	67	2	0.425
Dry matter (g/kg)	245	247	249	1.8	0.686
Intramuscular fat (g/kg)	23.2	19.4	19.0	1.1	0.232
Cholesterol (mg/kg)	747	774	727	13	0.280

Eight pigs per treatment. Analyses were carried out in duplicate

Table 3. Oxidative stability* of meat and subcutaneous fat of pigs fed the control diet or diets containing linseeds or CLA-oil

Sample		Diet			SEM	*P*
		control	linseed	linseed + CLA		
Meat	Day 1	0.037^{a}	0.104^{b}	0.084^{b}	0.016	0.001
	Day 3	0.065^{a}	0.139^{b}	0.109^{ab}	0.025	0.002
	Day 6	0.104	0.200	0.162	0.029	0.061
Backfat	Day 1	0.263^{a}	0.563^{ab}	0.604^{b}	0.068	0.037
	Day 3	0.713	1.201	1.197	0.106	0.052
	Day 6	1.211^{a}	1.790^{ab}	1.937^{b}	0.175	0.046

Eight pigs per treatment. Analyses were carried out in duplicate
*TBARS (malondialdehyde, mg/kg)
^{a,b}values within the same row not sharing the same superscripts differ significantly at $P < 0.05$

Dietary supplementation with linseed increased the percentage of linolenic acid and *trans*-10, *cis*-12 CLA in meat ($P < 0.05$). Supplementation with linseed and CLA decreased proportions of total MUFA in meat fatty acids and increased proportions of both main CLA isomers. Dietary supplementation with linseed increased the proportion of linolenic acid in backfat fatty acids ($P < 0.05$; Table 4). Supplementation with linseed and CLA increased CLA proportion in backfat fatty acids, the proportion of SFA, the proportion of total PUFA and reduced the proportion of total MUFA ($P < 0.05$).

Table 4. Fatty acid profile (mg per g of fatty acids determined) of meat and backfat of pigs fed the control diet or diets containing linseeds or CLA-oil

	Diet			SEM	*P*
	control	linseed	linseed + CLA		
Meat					
Saturated fatty acids	361.9	340.1	363.8	8.2	0.384
Monounsaturated fatty acids	504.0^{a}	474.3^{a}	430.2^{b}	10.2	0.001
Polyunsaturated fatty acids					
Linolenic acid	5.8^{a}	20.2^{b}	23.5^{b}	3.4	0.007
CLA (*c*9, *t*11)	1.5^{a}	7.4^{ab}	12.4^{b}	2.5	0.039
CLA (*t*10, *c*12)	0.1^{a}	6.0^{b}	8.9^{b}	1.4	0.055
Total PUFA	134.1	185.6	206.0	14.4	0.060
Backfat					
Saturated fatty acids	399.3^{a}	399.0^{a}	452.3^{b}	7.7	0.000
Monounsaturated fatty acids	457.3^{a}	442.7^{a}	335.6^{b}	13.1	0.000
Polyunsaturated fatty acids					
Linolenic acid	14.6^{a}	18.5^{b}	22.7^{b}	1.2	0.016
CLA (*c*9, *t*11)	4.6^{a}	2.3^{a}	26.5^{b}	2.6	0.000
CLA (*t*10, *c*12)	3.6^{a}	1.1^{a}	16.9^{b}	1.7	0.000
Total PUFA	144.3^{a}	158.3^{a}	212.1^{b}	8.2	0.000

Eight pigs per treatment. Analyses were carried out in duplicate
^{a,b}values in the same row not sharing the same superscripts differ ($P < 0.05$)

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Table 5. CLA isomers (mg per 100 g of fresh tissue) of meat and backfat in pigs fed the control diet or diets containing linseeds or CLA-oil*

Sample	Diet	CLA isomers					
		*c*9 *t*11	*t*10 *c*12	Σ*tt*	Σ*cc*	other *ct*	total CLA
Meat	control	3.24[a]	2.00[a]	0.17[a]	0.55	0.21	6.17[a]
	linseed	6.49[a]	4.84[a]	0.19[a]	0.58	0.46	12.56[a]
	linseed + CLA	21.70[b]	15.31[b]	0.59[b]	0.6	0.61	38.81[b]
	SEM	1.62	1.28	0.08	0.06	0.46	3.96
	diet (*P*)	0.081	0.188	0.033	0.894	0.572	0.158
Backfat	control	167.9[a]	100.9[a]	4.1[a]	6.6[a]	2.7[a]	282.2[a]
	linseed	126.4[a]	48.1[a]	4.3[a]	5.2[a]	3.8[a]	187.9[a]
	linseed + CLA	1190.3[b]	626.7[b]	38.1[b]	16.6[b]	33.3[b]	1905.0[b]
	SEM	120.3	65.1	4	1.4	7.2	191.8
	diet (*P*)	0	0	0	0	0	0

Eight pigs per treatment. Analyses were carried out in duplicate

*CLA-oil contained CLA isomers *c*9 *t*11, *t*10 *c*12, Σ*tt*, Σ*cc* and other *ct* at 498, 477, 3.6, 10.8 and 10.6 mg/g total CLA, respectively

[a,b]values in the same column and section with different superscripts differ significantly ($P < 0.05$)

Supplementation with CLA significantly increased the content of total CLA and isomers *cis*-9, *trans*-11 CLA, *trans*-10, *cis*-12 CLA, and *trans*-, *trans*-CLA in meat (Table 5). Dietary CLA significantly increased the content of total CLA and all CLA isomers in the backfat. The *cis*-9, *trans*-11 isomer was found to be 41.7% and 89.9% more concentrated in meat and backfat, respectively, than the *trans*-10, *cis*-12 isomer. All CLA isomers were present also in meat and backfat of pigs fed CLA-free diets.

DISCUSSION

The Prestice Black-Pied pig is a Czech autochtonous breed from the western region of Bohemia. This breed is reared in a closed population which is included in the National programme for the conservation and use of genetic resources. The breed, as other unimproved breeds, is characterised by a strong constitution, good maternity traits, lower daily gain and higher carcass fatness in comparison with modern crossbreeds (Dostalova et al. 2012). The importance of this breed lies in its good reproductive performance, adaptability, vitality and resistance to diseases (Lustykova et al. 2008).

In the present study neither linseed nor linseed combined with CLA influenced the tested meat quality parameters. No effect of linseed on carcass value and meat quality was observed in our previous experiment (Vaclavkova et al. 2014). Van Oeckel et al. (1997), however, reported that the lean meat percentage was lower in pigs fed the highest linseed level (5.4%). Reports on the effects of dietary CLA on fat deposition in pigs are not consistent. Dugan et al. (1997) reported that pigs fed CLA deposited less subcutaneous fat and gained more lean meat irrespective of sex. Thiel-Cooper et al. (2001) demonstrated that CLA led to less intramuscular and subcutaneous fat. However, Muller et al. (2000) did not observe any difference in fat deposition between control and CLA-fed pigs. Dugan et al. (1999) reported that feeding 2% CLA to pigs had the potential to improve pork composition by increasing intramuscular fat, which was also observed by Cordero et al. (2010). Martin et al. (2008c) reported no effect of CLA at 1 or 2% on backfat thickness and concluded that the effect of CLA on increasing intramuscular fat might depend on the level of CLA supplementation. In our experiment, the backfat thickness was not significantly affected by the diet. Feeding linseed and the combination of linseed with CLA led to a non-significant decrease of intramuscular fat. The intramuscular fat concentration of meat from pigs fed experimental diets was lower than 2 to 3%, which was reported to be the optimal fat content for pork of eating quality (Verbeke et al. 1999).

Oxidation of unsaturated fat is responsible for deterioration of flavour and aroma, and decreased

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shelf life of meat and backfat of pigs fed oilseeds. Karolyi et al. (2012) compared lipid oxidation of meat of pigs fed a control diet and a diet containing 3% linseed. The oxidation of meat lipids measured as TBARS did not differ between the two groups of pigs after cold storage for up to six days, but levels of the same were elevated in the backfat of linseed-fed pigs already after three days of storage. D'Arrigo et al. (2002) showed that the adipose tissue from pigs fed diets supplemented with linseed oil was more susceptible to oxidation than that from pigs fed diets containing sunflower oil. Effects of CLA on the oxidative stability of porcine fat are ambiguous. Martin et al. (2008a) reported that dietary CLA added at 2% to a commercial diet of gilts increased TBARS values of loin meat after seven days of refrigerated storage. In another experiment, CLA supplementation (1 or 2%) decreased peroxidation values in liver, but not in the loin (Martin et al. 2008b). In the present study, linseed supplementation resulted in higher peroxidation of tissue lipids. Dietary CLA did not influence production of aldehydes during storage of meat and backfat.

Dietary effects of linseed and CLA were more pronounced in backfat than in meat. CLA was incorporated into lipids of both tissues. The *cis*-9, *trans*-11 CLA isomer was incorporated more efficiently into tissues than the *trans*-10, *cis*-12 isomer, which was observed also by other authors (Bee 2001; Lauridsen et al. 2005; Han et al. 2011). CLA supplementation significantly reduced the proportion of MUFA and increased SFA in meat and backfat fatty acids. Both effects are well known, and are caused by the inhibitory effect of CLA on activity of Δ9 desaturase (Hur et al. 2007).

The concentration of total CLA (in mg/100 g of fresh tissue) in backfat was almost fifty times higher than in meat. It is possible that CLA has no important physiological role in muscle tissue and represents a mere energy reserve deposited in the adipose tissue. Several findings are consistent with this hypothesis. Dietary CLA was found to not influence serum hormones and metabolites (Stangl et al. 1999; Ramsay et al. 2001). Dietary CLA was observed to not change the profile of lipoproteins (Stangl et al. 1999). In pigs fed CLA the CLA/total PUFA ratio was higher in muscle neutral lipids than in polar lipids (Martin et al. 2008b; Tous et al. 2013). This suggests a limited uptake of CLA for synthesis of membranes. CLA-oil contained 4.4% more *cis*-9, *trans*-11 CLA than *trans*-10, *cis*-12 CLA. In lipids of meat and backfat, however, the former isomer was present at clearly higher concentrations, which was observed also by other authors (e.g. Han et al. 2011). Both meat and backfat of pigs fed CLA-free diets contained CLA at significant concentrations. The CLA content of pork reported in several studies varied from 0.6 to 1.5 mg/g of fat (reviewed by Schmid et al. 2006). The presence of CLA in pig meat may be the result of microbial conversion of linoleic acid to CLA in the intestine. This microbial activity has been reported in lactobacilli (Macouzet et al. 2009), *Butyrivibrio fibrisolvens* (Fukuda et al. 2005), and may be characteristic of a range of intestinal bacteria.

It can be concluded that linseed supplementation increased the susceptibility of meat and backfat lipids to oxidation, which was not reduced by dietary CLA. Dietary CLA changed the fatty acid profile of meat and backfat; however, its effects on the lean percentage, intramuscular fat and other meat quality parameters were negligible.

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