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The influence of dietary linseed on alpha-linolenic acid and its longer-chain n-3 metabolites content in pork and back fat

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ABSTRACT

The aim of this study was to examine the influence of dietary linseed on fatty acid profile and lipid oxidation in pig muscle (m. longissimus dorsi) and back fat. Sixty PIC fatteners were equally allotted to dietary treatments at an initial live weight of about 27 kg and fed for 90 days with a control or a linseed diet (3% linseed, 105 mg/kg of α-tocopheryl acetate). Growth performance, carcass traits and meat quality were not affected by feeding linseed to any significant extent. No differences were found in the proportion of saturated, monounsaturated and polyunsaturated fatty acids of muscle or back fat between diets. However, both muscle and back fat from the linseed diet had a significantly higher n-3 and polyunsaturated fatty acid proportion (%), including C18:3n-3, C20:5n-3 and C22:6n-3, and a lower n-6/n-3 ratio compared with the control diet (4.3 vs.1.3, 1.9 vs. 0.44, 0.76 vs. 0.14, 0.17 vs. 0.12, and 4.6 vs. 17.4 ratio in muscle, and 5.3 vs.1.2, 4.3 vs. 0.87, 0.07 vs. 0.02, 0.04 vs. 0.02, and 2.8 vs. 15.8 ratio in back fat, respectively). Lipid oxidation, measured as 2-thiobarbituric acid-reactive substances, was similar in muscle when feeding both diets after cold storage up to 6 days, but more developed in back fat when feeding the linseed diet after 3 days of cold storage. This study confirmed that continuous feeding of pigs with a relatively low level of linseed can produce a pork meat enhanced with both C18:3n-3 and pre-formed C20-22n-3, and with nutritionally optimized n-6/n-3 ratios, without adverse effects on fatteners' performances, carcass traits and meat quality.

Key words: pork, back fat, n-3 fatty acids, lipid oxidation, linseed

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Introduction

Among the strategies for developing healthier meat, the modification of its fatty acid (FA) composition has become increasingly important in recent years. Particularly n-3 polyunsaturated fatty acids (PUFA), i.e. α-linolenic acid (ALA, C18:3n-3) and its longer-chain metabolites eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) are receiving considerable interest as they are of particular relevance to human health, and the enrichment of meat with n-3 PUFA could help bridge the gap between their recommended and actual intake (GIVENS et al., 2006). Pork meat has great potential in this regard as it is among the most consumed meats worldwide, and its FA composition can be modulated easily by dietary means (PETTIGREW and ESNAOLA, 2001). It is well established that the FA composition of pork is influenced by the FA composition of dietary fat (WOOD et al., 2003). When fed to pigs, ALA is absorbed practically unchanged, and incorporated into adipose tissue and cell membranes, where desaturation and chain elongation processes may occur. Hence, feeding with ALA sources such as linseed, have been proven to be very efficient in increasing the n-3 PUFA content of pig fat depots in the form of ALA (ROMANS et al., 1995; ENSER et al., 2000; RILEY et al., 2000; KOUBA et al., 2003; CORINO et al., 2008; HAAK et al., 2008; HUANG et al., 2008; GUILLEVIC et al., 2009). Conversion of ALA into longer-chain n-3 PUFA is however much less significant due to the complex nature of the enzymatic system involved, consisting of desaturases and elongases (RAES et al., 2004). Nevertheless, the ALA to C20-22n-3 conversion processes may be, to some extent, influenced by the level of linseed supplementation and starting weight/age of the pig. These factors seem to particularly affect the *in vivo* synthesis of DHA, which has only been increased in a few previous studies after feeding relatively low levels (~2-5%) of dietary linseed (ENSER et al., 2000; CORINO et al., 2008).

The aim of the present study was, therefore, to feed 3% linseed diet to pigs throughout the fattening period and investigate the incorporation of ALA and its longer-chain n-3 metabolites in muscle and back fat. The growth performance, carcass traits, meat quality and susceptibility to lipid oxidation in muscle and back fat were also investigated.

Materials and methods

The experiment was conducted at a commercial pig farm in compliance with the national legislation on animal protection (ANONYM., 2006). Sixty PIC fatteners (barrows and gilts) were equally allotted to dietary treatments at initial live weight of about 27 kg and fed during the fattening period (90 d) with control (C) or linseed (L) diet *ad libitum*. The diet ingredients and calculated nutritive values (ANONYM., 1998a) are shown in Table 1.

Table 1. Ingredients and calculated nutritive value of the diets

Diet	Control	Linseed
Ingredients (% in diet)		
Corn	45.00	45.00
Triticale	15.00	15.00
Soybean meal (44%)	23.56	21.30
Wheat	7.30	6.00
Linseed	-	3.00
Wheat bran	5.00	5.00
Beet molasses	0.50	1.00
Calcium carbonate	1.40	1.40
Mono calcium phosphate	1.10	1.10
Sodium chloride	0.45	0.45
DL-methionine (98%)	0.03	0.03
L-lysine (78.8%)	0.16	0.22
Premix[1]	0.50	0.50
Calculated nutritive value[2]		
Dry matter (%)	89.1	89.1
Crude protein (%)	18.3	17.8
Crude fat (%)	3.3	3.4
Metabolizable energy (MJ/kg)	12.5	12.4

[1]Premix provided per kg of diet: vitamin A 7000 I.U., vitamin D3 800 I.U., vitamin E (as α-tocopheryl acetate) 55 mg[†], vitamin B1 2.6 mg, vitamin B2 4 mg, vitamin B6 3.5 mg, vitamin B12 23 mcg, pantothenic acid 14 mg, nicotinic acid 23 mg, biotin 0.06 mg, folic acid 0.6 mg, choline 200 mg, vitamin K3 3 mg, Fe 95 mg, Cu 20 mg, Mn 50 mg, Zn 100 mg, Co 0.4 mg, Ca 0.5 mg and Se 0.3 mg. [†]Linseed diet was additionally supplemented with 50 g of α-tocopheryl acetate per kg. [2]On the basis of composition of feedstuffs (ANONYM., 1998a)

At the end of the trial, the pigs were humanely slaughtered at a commercial slaughterhouse using the standard procedure. The pH was measured by the penetration electrode of a pH meter (Testo 230, Testo, Germany) on the right side of the carcass in the m. longissimus dorsi (MLD) at the level of the last rib at 45 min (pH1) and 24 h (pH24) *post mortem*. Meat colour was measured at 24 h *post mortem* on the fresh cut surface of MLD at the level of the last rib by chroma meter (CR-410, KONICA MINOLTA, Japan) after 5 min. of bloom time. The results for *CIE L** (lightness) were reported.

The samples of MLD and back fat (BF) were taken at the level of the last rib and stored frozen (-20 ºC) until analyses of chemical composition (of MLD only), FA composition and lipid oxidation. The moisture, ash, protein and fat content were determined by

standard methods for the meat and meat products group (ANONYM., 1997, 1998b, 1978 and 2001, respectively). The FA composition was determined by gas liquid chromatography using the *in situ* transesterification method (PARK and GOINS, 1994). The content of FA methyl esters (FAME) was determined using an Agilent Technologies 6890 N (USA) gas chromatograph equipped with a flame ionisation detector and the capillary column Supelco Omegawax™ 320 (length 30 m, internal diameter 0.32 mm and film thickness 0.25 µm) for FAME separation. Separated FAMEs were identified by comparison with the retention times of the FAMEs in a standard mixture (Nu-Check Prep, Inc, Elysian, USA). The same standard mixture was used to determine the response factor (Rf) for each FA. The mass portion of each FA in the sample was determined using the Rf and the factor of conversion of FA content from the FAME content.

To measure the lipid oxidation, the thawed samples of MLD and BF were homogenised, wrapped in an oxygen permeable polyethylene film and analyzed after 0, 3 and 6 days (for MLD), or after 0 and 3 days (for BF) of cold storage at 4 °C. Lipid oxidation was measured by assaying the 2-thiobarbituric acid-reactive substances (TBARS) in samples using the method of BOTSOGLOU et al. (1994), slightly modified. Briefly, 2 g of sample was weighed into a 50 mL PP test tube, and 8 mL of 5% aqueous trichloroacetic acid (TCA) and 5 mL of 0.8% butylated hydroxytoluene (BHT) in hexane was added to each tube. The content was homogenized (Ika T10 basic, Ultra Turrax, Germany) for 30 s at high speed, and then centrifuged for 5 min at 2500 g (Centric 322A, Tehtnica, Slovenia). The upper hexane layer was discarded. The bottom aqueous layer was made up to 10 mL volume with 5% TCA. If necessary, the reaction mixtures were filtered (grade 391; Munktell, Germany). A 2.5 mL aliquot was pipetted into a screw-capped PP tube, a 1.5 mL of 0.8% aqueous 2-thiobarbituric acid (TBA) was added, and the content was incubated for 30 min at 95 °C (Memmert, Germany). After incubation, the tubes were cooled under tap water, and absorbance at 532 nm was determined (Helios γ, Thermo Electron Corporation, UK) against a blank containing 2.5 mL of TCA and 1.5 mL of 0.8% aqueous TBA. The content of malonaldehyde (MDA) was calculated from the standard curve using 1,1,3,3 - tetrametoxypropane. The TBARS were measured in triplicate and expressed as mg of MDA per kg of tissue.

Data from both diets were compared by Student's *t*-test.

Results

The effect of diets on growth performance, carcass and meat quality traits is shown in Table 2. No differences were found ($P>0.05$) in the final weight, average daily gain, carcass traits and chemical composition or pH of MLD between the diets, except for MLD colour L^* value, which was slightly higher ($P\leq0.05$) in the C than in the L diet.

Fatty acid composition of MLD and BF of pigs fed C or L diet is shown in Table 3 and 4, respectively. There was no difference (P>0.05) between the diets in the proportion of SFA, MUFA and PUFA and P/S ratio in MLD (Table 3). However, the L diet increased (P≤0.01) the proportion of all individual n-3 PUFA, including ALA, EPA and DHA, and decreased (P≤0.01) the n-6 PUFA proportion and n-6/n-3 ratio in MLD compared with the C diet. The proportion of C14:0 and C16:1 in BF (Table 4) was lower (P≤0.01) in the L than in the C diet; however, no differences were found (P>0.05) in total SFA and MUFA, as well as PUFA and P/S ratio. The L diet resulted again in higher (P≤0.01) proportions of all individual n-3 PUFA, and a lower (P≤0.01) n-6 PUFA proportion and n-6/n-3 ratio compared with the C diet.

Table 2. Growth performances, carcass and meat quality of pigs fed control or linseed diet

Diet	Control (n = 30)	Linseed (n = 30)
Initial weight (kg)	26.9 ± 1.4	26.7 ± 1.4
Average daily gain[1] (kg)	0.84 ± 0.13	0.80 ± 0.09
Final weight (kg)	102.9 ± 12.7	99.0 ± 8.4
Hot carcass weight (kg)	79.5 ± 10.4	77.3 ± 6.6
Killing-out (%)	77.2 ± 2.3	78.0 ± 1.5
M (mm)	67.0 ± 5.8	65.1 ± 5.1
S (mm)	11.0 ± 5.2	12.5 ± 4.2
M (%)	59.5 ± 4.4	57.4 ± 3.8
MLD pH1	6.4 ± 0.19	6.4 ± 0.21
MLD pH24	5.7 ± 0.10	5.7 ± 0.07
MLD colour L* value	57.1[a] ± 2.7	55.8[b]± 1.7
MLD Water (%)	74.1 ± 0.78	74.3 ± 0.59
MLD Ash (%)	1.14 ± 0.03	1.13 ± 0.02
MLD Protein (%)	23.9 ± 0.71	23.7 ± 0.65
MLD IMF (%)	1.2 ± 0.38	1.4 ± 0.31

Means ± S.D.; [a,b] Row means with different superscripts differ significantly at P≤0.05; [1]calculated as (final weight-initial weight)/number of feeding days; M = shortest distance between the cranial end of m. glutaeus medius and dorsal edge of spinal canal; S = fat thickness at its narrowest part over the m. glutaeus medius); M% = calculated lean meat percentage (ANONYM., 2007); MLD = m. longissimus dorsi; IMF = intramuscular fat.

Table 3. Fatty acid (FA) composition of m. longissimus dorsi of pigs fed control or linseed diet

Diet	Control (n = 30)	Linseed (n = 30)
g/100g of total FA		
C14:0	1.1 ± 0.16	1.1 ± 0.18
C16:0	21.4 ± 1.3	21.5 ± 1.2
C16:1	2.63 ± 0.45	2.57 ± 0.45
C18:0	14.7 ± 2.0	14.3 ± 2.0
C18:1	33.7 ± 4.3	35.2 ± 3.0
C18:2n-6	15.2^{A} ± 2.6	13.5^{B} ± 1.9
C18:3n-3 (ALA)	0.44^{B} ± 0.09	1.9^{A} ± 0.34
C20:1	0.51 ± 0.09	0.52 ± 0.07
C20:2n-6	0.37^{A} ± 0.12	0.29^{B} ± 0.05
C20:3n-6	0.57 ± 0.12	0.52 ± 0.09
C20:4n-6	4.8^{A} ± 1.3	3.4^{B} ± 0.88
C20:3n-3	0.08^{B} ± 0.02	0.28^{A} ± 0.04
C20:5n-3 (EPA)	0.14^{B} ± 0.04	0.76^{A} ± 0.20
C22:4n-6	0.79^{A} ± 0.20	0.40^{B} ± 0.14
C22:5n-3	0.55^{B} ± 0.16	1.2^{A} ± 0.27
C22:6n-3 (DHA)	0.12^{B} ± 0.04	0.17^{A} ± 0.06
Σ SFA	38.3 ± 1.4	37.8 ± 1.6
Σ MUFA	38.4 ± 4.2	39.7 ± 3.0
Σ PUFA	23.3 ± 4.4	22.5 ± 3.3
Σ n-6	21.2^{A} ± 4.2	18.2^{B} ± 2.9
Σ n-3	1.3^{B} ± 0.28	4.3^{A} ± 0.84
n-6/n-3	17.4^{A} ± 1.5	4.6^{B} ± 2.7
P/S	0.61 ± 0.13	0.60 ± 0.10

Means ± S.D.; ^{A,B} Row means with different superscripts differ significantly at P≤0.01; ALA - alpha-linolenic acid; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; Σ SFA - saturated fatty acids = (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0); Σ MUFA - monounsaturated fatty acids = (C12:1 + C16:1 + C17:1 + C18:1 + C20:1); Σ PUFA - polyunsaturated fatty acids = (C18:2n-6 + C18:3n-6 + C18:2n -7 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:4n-6 + C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); P/S = PUFA/SFA

Table 4. Fatty acid (FA) composition of back fat of pigs fed control or linseed diet

Diet	Control (n = 30)	Linseed (n = 30)
g/100g of total FA		
C14:0	$1.4^{A} \pm 0.13$	$1.3^{B} \pm 0.10$
C16:0	23.3 ± 1.5	22.6 ± 2.0
C16:1	$2.0^{A} \pm 0.37$	$1.7^{B} \pm 0.21$
C18:0	13.6 ± 1.9	13.7 ± 1.9
C18:1	37.6 ± 2.7	38.3 ± 2.4
C18:2n-6	$16.5^{A} \pm 3.1$	$13.6^{B} \pm 1.9$
C18:3n-3 (ALA)	$0.87^{B} \pm 0.15$	$4.3^{A} \pm 0.52$
C20:1	0.82 ± 0.15	0.82 ± 0.12
C20:2n-6	$0.70^{a} \pm 0.14$	$0.63^{b} \pm 0.09$
C20:3n-6	$0.14^{a} \pm 0.10$	$0.09^{b} \pm 0.01$
C20:4n-6	$0.30^{A} \pm 0.08$	$0.21^{B} \pm 0.04$
C20:3n-3	$0.14^{B} \pm 0.04$	$0.64^{A} \pm 0.06$
C20:5n-3 (EPA)	$0.02^{B} \pm 0.02$	$0.07^{A} \pm 0.01$
C22:4n-6	$0.15^{A} \pm 0.04$	$0.07^{B} \pm 0.01$
C22:5n-3	$0.09^{B} \pm 0.02$	$0.27^{A} \pm 0.04$
C22:6n-3 (DHA)	$0.02^{B} \pm 0.01$	$0.04^{A} \pm 0.01$
Σ SFA	39.7 ± 3.2	38.5 ± 3.8
Σ MUFA	41.1 ± 3.0	41.4 ± 2.6
Σ PUFA	19.2 ± 3.6	20.1 ± 2.6
Σ n-6	$17.8^{A} \pm 3.3$	$14.6^{B} \pm 2.0$
Σ n-3	$1.2^{B} \pm 0.21$	$5.3^{A} \pm 0.59$
n-6/n-3	$15.8^{A} \pm 0.83$	$2.8^{B} \pm 0.25$
P/S	0.49 ± 0.12	0.53 ± 0.14

Means ± S.D.; ^{a,b} or ^{A,B} Row means with different superscripts differ significantly at P≤0.05 or P≤0.01, respectively; ALA - alpha-linolenic acid; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; Σ SFA - saturated fatty acids = (C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0); Σ MUFA - monounsaturated fatty acids = (C12:1 + C14:1 + C16:1 + C17:1 + C18:1 + C19:1 + C20:1 + C22:1); Σ PUFA - polyunsaturated fatty acids = (C18:2n-6 + C18:3n-6 + C18:2n-7 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:2n-6 + C22:4n-6 + C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3); P/S = PUFA/SFA.

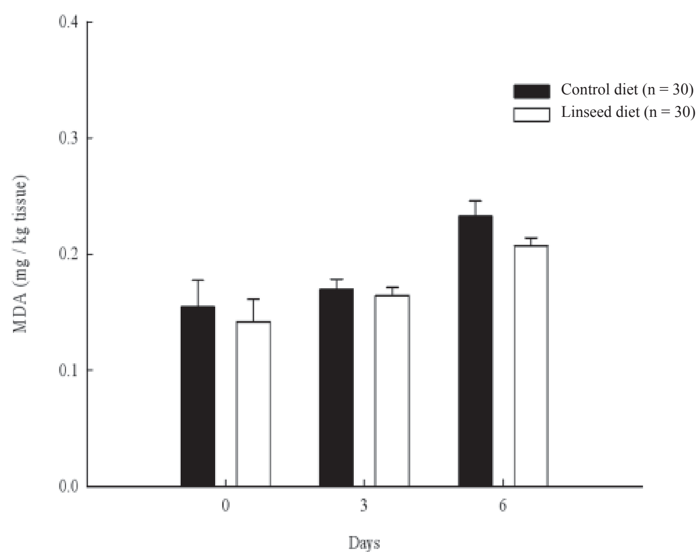

Fig. 1. Thiobarbituric acid-reactive substance (TBARS) values of m. longissimus dorsi (means with standard errors indicated as error bars); expressed in mg of malonaldehyde (MDA) per kg.

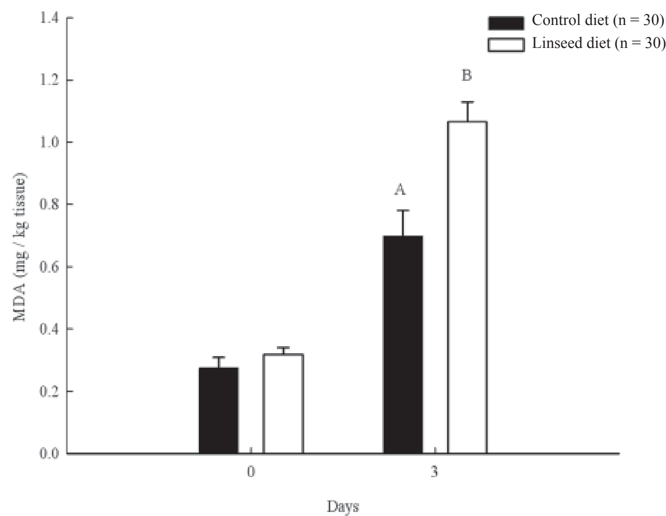

Fig. 2. Thiobarbituric acid-reactive substance (TBARS) values of back fat (means with standard errors indicated as error bars); expressed in mg of malonaldehyde (MDA) per kg; A, B = P≤0.01.

Results for lipid oxidation measured by TBARS values of MLD and BF are shown in Fig. 1 and Fig. 2, respectively. The lipid oxidation of MLD measured during cold storage at days 0, 3 and 6 did not differ ($P>0.05$) between the diets. The initial lipid oxidation of BF was similar ($P>0.05$) for both diets, but after 3 days of cold storage it was more developed ($P\leq0.01$) when the L diet was fed.

Discussion

The results of the present study agree with several previous reports where linseed diet did not affect pig performance, carcass traits and meat quality, but had a significant impact on FA profile (ROMANS et al., 1995; RILEY et al., 2000; KOUBA et al., 2003; CORINO et al., 2008; GUILLEVIC et al., 2009). Feeding pigs with 3% linseed diet for 90 days in the present study increased the ALA proportion in both MLD and BF more than 4-fold compared to the control. The proportion of ALA was much greater in the subcutaneous than in the intramuscular fat, which is consistent with previous findings (ENSER et al., 2000; RILEY et al., 2000; KOUBA et al., 2003; CORINO et al., 2008). In addition, long-term feeding with relatively low linseed levels in the present study significantly increased the proportion of all long-chain n-3 PUFA, including an increase in EPA and DHA by more than 5-fold and about 1.4-fold in MLD, and 3.5-fold and 2-fold in BF, respectively, as compared to control. The C20-22n-3 proportions were higher in the muscle than in the adipose tissue, which was also observed by ENSER et al. (2000), KOUBA et al. (2003) and CORINO et al. (2008). It appears that factors such as the starting weight/age of the pig and the level of linseed supplementation may affect the incorporation of long-chain n-3 PUFA into the tissues. For instance, the strategy of short-term pre-slaughter feeding with higher linseed level, e.g., 11.4% during the last 24 days (RILEY et al., 2000) or 15% during the last 28 days (ROMANS et al., 1995), was less effective at increasing the C20-22n-3 content than longer-term feeding with lower linseed levels. The PUFA are mostly incorporated into the membrane phospholipids. The composition of the membranes is more subject to changes in earlier than in later stages of life, thereby allowing more efficient incorporation of long-chain n-3 PUFA in younger than in more mature animals (RAES et al., 2004). Besides, competition between ALA and C20-22n-3 for incorporation into the structural lipids may exist when the concentration of dietary ALA is high (RILEY et al., 2000). This competition appears to be particularly evident for DHA, the level of which in a pig's tissues was not increased by linseed feeding in several previous studies (RILEY et al., 2000; KOUBA et al., 2003; HAAK et al., 2008; GUILLEVIC et al., 2009). The formation of DHA is controlled by complex enzyme systems, which can convert C22:5n-3 into DHA either directly by the action of Δ-4 desaturese, or via the alternative pathway proposed by SPRECHER et al. (1995), consisting of C22:5n-3 chain elongation and subsequent Δ-6 desaturation of C24:5n-3 and β-oxidation of C24:6n-3. Hence, the lack of effect on DHA may be explained by competition for Δ-6 desaturase activity between dietary ALA and C24:5n-3.

The competition between DHA and other C20-22 n-3 and/or the low metabolic activity of desaturases and elongases could also be responsible for inhibiting DHA increase, as suggested by RAES et al. (2004).

The increased DHA proportions observed in the present study are consistent with the results of ENSER et al. (2000), who reported a small but significant increase in DHA in pig muscle and adipose tissue after long-term feeding with a diet containing only 0.4% ALA from linseed. As the authors speculated, the small but constantly elevated levels of ALA in the diet probably minimized the possibility of ALA-DHA competition, and such an explanation would also be relevant for the present results. On the other hand, several recent studies reported the quite variable effects of various dietary linseed regimes on the level of DHA in the pigs' tissues. For instance, DHA was increased in muscle but not in the back fat with an increase in time on a high (10%) linseed diet in the study of HUANG et al. (2008). JUÁREZ et al. (2010) also found no effect of duration (4, 8 and 12 weeks) and level (5, 10 and 15%) of linseed feeding on DHA in back fat. On the contrary, CORINO et al. (2008) reported an increase in DHA in both the muscle and back fat of pigs fed with 5% linseed diet during approx. final third of fattening. However, lower linseed levels and longer feeding durations, i.e. 4.2% and 3% of dietary linseed fed to pigs for the second half or continuously throughout fattening in the studies of GUILLEVIC et al. (2009) and HAAK et al. (2008), respectively, had no influence on DHA level in muscle. These inconsistencies may indicate the possible influence of other factors; e.g., the proportion of n-3 PUFA deposited in tissues as a result of dietary ALA to C20-22 n-3 conversion was found to be affected by the genotype of the pigs (KLOAREG et al., 2007).

As expected, the MLD and BF of control pigs in the present study showed typically high n-6/n-3 ratios (17.42 and 15.75, respectively). On the contrary, an increase in the n-3 PUFA proportion, together with the n-6 PUFA decrease, produced a large and nutritionally beneficial reduction in the n-6/n-3 ratio in both the MLD and BF of linseed fed pigs (4.63 and 2.75, respectively).

It is well-known that an increase in n-3 PUFA may decrease the oxidative stability of pork and impair its sensory properties. To control the lipid oxidation in the present study, an additional antioxidant was added to the L diet compared to the C diet (105 mg vs. 55 mg of α-tocopheryl acetate per kg, respectively). The proportion of ALA in the MLD, although significantly increased by the L diet, was below the 3% threshold at which abnormal pork flavour may occur (WOOD et al., 2003). In addition, oxidative stability of MLD was not affected by the L diet. In fact, all TBARS values in MLD were well below the threshold of 0.5 mg of MDA per kg, at which consumers are likely to detect off-flavours in meat (GRAY and PEARSON, 1987).

Less than 23% PUFA in BF is recommended for finishing pigs to help reduce oxidative problems (BRYHNI et al., 2002). In the present study the PUFA proportions in BF did not differ between diets and were generally below that maximum. On the other hand,

the initial TBARS values in BF were higher than those observed in MLD, regardless of the diet. Additionally, there was a significant decrease in oxidative stability of BF from pigs fed the L diet after 3 days of cold storage. This was probably due to much greater ALA deposition in those animals. Vitamin E is fat-soluble and is deposited in both cell membranes and adipose cells (MILLER, 2002). However, it seems that at the dose used in the present study, the deposition of vitamin E was not sufficient to prevent oxidation to the same extent in both the analysed tissues. For additional oxidative stability of BF, therefore, a higher dietary level of antioxidant should be investigated.

In conclusion, this study confirmed that continuous feeding of pigs with a relatively low level of linseed can produce a pork meat enhanced with beneficial n-3 PUFA, with nutritionally optimized n-6/n-3 ratios and without adverse effects on fatteners' performances and meat quality. Linseed diet increased the tissue proportions of both ALA and its longer-chain n-3 metabolites, which supports the fact that pigs can synthesize DHA *in vivo* when fed low levels of dietary ALA. In this context, linseed-fed pork has great potential to deliver more ALA and pre-formed long-chain n-3 PUFA into the modern diet.

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References

ANONYMOUS (1978): Meat and meat products group - Determination of nitrogen content. ISO Standard No 937. International Organization for Standardization, Geneva, Switzerland.

ANONYMOUS (1997): Meat and meat products group - Determination of moisture content. ISO Standard No 1442. International Organization for Standardization, Geneva, Switzerland.

ANONYMOUS (1998a): Nutrient Requirements of Domestic Animals, Nutrient Requirements of Swine, 10th revised Edition, National Research Council, Washington, DC, USA.

ANONYMOUS (1998b): Meat and meat products group - Determination of total ash. ISO Standard No 936. International Organization for Standardization, Geneva, Switzerland.

ANONYMOUS (2001): Meat and meat products group - Determination of total fat content. ISO Standard No 1443. International Organization for Standardization, Geneva, Switzerland.

ANONYMOUS (2006): Law on animal protection (in Croatian). Narodne Novine 135/06.

ANONYMOUS (2007): Regulations on the quality of pig carcasses at slaughter (in Croatian). Narodne Novine 40/07, Appendix 3.

BOTSOGLOU, N. A., D. J. FLETOURIS, G. E. PAPAGEORGIU, V. N. VASSILOPOULOS, A. J. MANTIS, A. G. TRAKATELLIS (1994): Rapid, sensitive, and specific thiobarbituric acid

method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J. Agr. Food Chem. 42, 1931-1937.

BRYHNI, E. A., N. P. KJOS, R. OFSTAD, M. HUNT (2002): Polyunsaturated fat and fish oil in diet for growing-finishing pigs: effects on fatty acid composition and meat, fat, and sausage quality. Meat Sci. 62, 1-8.

CORINO, C., M. MUSELLA, J. MOUROT (2008): Influence of extruded linseed on growth, carcass composition, and meat quality of slaughtered pigs at one hundred ten and one hundred sixty kilograms of live weight. J. Anim. Sci. 86, 1850-1860.

ENSER, M., R. I. RICHARDSON, J. D. WOOD, B. P. GILL, P. R. SHEARD (2000): Feeding linseed to increase the n-3 PUFA of pork: Fatty acid composition of muscle, adipose tissue, liver and sausages. Meat Sci. 55, 201-212.

GIVENS, D. I., K. E. KLIEM, R. A. GIBBS (2006): The role of meat as a source of n-3 polyunsaturated fatty acids in the human diet. Meat Sci. 74, 209-218.

GRAY, J. I., A. M. PEARSON (1987): Rancidity and warmed-over flavour. In: Advances in meat research, vol. 3, restructured meat and poultry products. (Pearson, A. M., T. R. Dutson, Eds.) Van Nostrand Reinhold Co, New York. pp. 221-269.

GUILLEVIC, M., M. KOUBA, J. MOUROT (2009): Effect of a linseed diet on lipid composition, lipid peroxidation and consumer evaluation of French fresh and cooked pork meats. Meat Sci. 81, 612-618.

HAAK, L., S. DE SMET, D. FREMAUT, K. VAN WALLEGHEM, K. RAES (2008): Fatty acid profile and oxidative stability of pork as influenced by duration and time of dietary linseed or fish oil supplementation. J. Anim. Sci. 86, 1418-1425.

HUANG, F. R., Z. P. ZHAN, J. LUO, Z. X. LIU, J. PENG (2008): Duration of dietary linseed feeding affects the intramuscular fat, muscle mass and fatty acid composition in pig muscle. Livest. Sci. 118, 132-139.

JUÁREZ, M., M. E. R. DUGAN, N. ALDAI, J. L. AALHUS, J. F. PATIENCE, R. T. ZIJLSTRA, A. D. BEAULIEU (2010): Feeding co-extruded flaxseed to pigs: Effects of duration and feeding level on growth performance and backfat fatty acid composition of grower-finisher pigs. Meat Sci. 84, 578-584.

KLOAREG, M., J. NOBLET, J. VAN MILGEN (2007): Deposition of dietary fatty acids, *de novo* synthesis and anatomical partitioning of fatty acids in finishing pigs. Brit. J. Nutr. 97, 35-44.

KOUBA, M., M. ENSER, F. M. WHITTINGTON, G. R. NUTE, J. D. WOOD (2003): Effect of high linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. J. Anim. Sci. 81, 1987-1979.

MILLER, R. K. (2002): Factors affecting the quality of raw meat. In: Meat processing - Improving quality. (Kerry, J., J. Kerry, D. Ledward, Eds.) Woodhead Publishing Ltd, Cambridge. pp. 27-63.

PARK, P. W., R. E. GOINS (1994): *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. J. Food Sci. 59, 1262-1266.

PETTIGREW, J. E., M. A. ESNAOLA (2001): Swine nutrition and pork quality: a review. J. Anim. Sci. 79, 316-342.

RAES, K., S. DE SMET, D. DEMEYER (2004): Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Anim. Feed Sci. Tech. 113, 199-221.

RILEY, P. A., M. ENSER, G. R. NUTE, J. D. WOOD (2000): Effects of dietary linseed on nutritional value and other quality aspects of pig muscle and adipose tissue. Anim. Sci. 71, 483-500.

ROMANS, J. R., D. M. WULF, R. C. JOHNSON, G. W. LIBAL, W. J. COSTELLO (1995): Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II. Duration of 15% dietary flaxseed. J. Anim. Sci. 73, 1987-1999.

SPRECHER, H., D. L. LUTHRIA, B. S. MOHAMMED, S. P. BAYKOUSHEVA (1995): Re-evaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J. Lipid Res. 36, 2471-2477.

WOOD, J. D., R. I. RICHARDSON, G. R. NUTE, A. V. FISHER, M. M. CAMPO, E. KASAPIDOU, P. R. SHEARD, M. ENSER (2003): Effects of fatty acids on meat quality: a review. Meat Sci. 66, 21-32.

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SAŽETAK

Cilj rada bio je istražiti utjecaj dodatka lana u hranidbi svinja na sastav masnih kiselina i oksidaciju lipida u mesu (m. longissimus dorsi) i leđnoj slanini. Šezdeset PIC nazimica i muških kastrata početne mase oko 27 kg ravnomjerno je bilo podijeljeno u dvije skupine i hranjeno tijekom tova (90 dana) uobičajenom krmnom smjesom ili krmnom smjesom s 3% sjemena lana i 105 mg/kg α-tokoferol acetata. Hranidba lanom nije značajnije utjecala na dnevni prirast, svojstva trupa i kakvoću mesa tovljenika. Između skupina nisu postojale značajnije razlike u sastavu zasićenih, jednostruko nezasićenih ili višestruko nezasićenih masnih kiselina mesa ili leđne slanine. Međutim, pri hranidbi lanom u odnosu na uobičajenu hranidbu utvrđen je značajno viši udio (%) n-3 masnih kiselina, uključujući C18:3n-3, C20:5n-3 i C22:6n-3, i niži n-6/n-3 omjer u mesu (4,3% prema 1,3%, 1,9% prema 0,44%, 0,76% prema 0,14%, 0,17% prema 0,12% i 4,6 prema 17,4) i leđnoj slanini (5,3% prema 1,2%, 4,3% prema 0,87%, 0,07% prema 0,02%, 0,04% prema 0,02%, i 2,8 prema 15,8). Oksidacija lipida, mjerena reaktivnim supstancijama na 2-tiobarbituratnu kiselinu, bila je podjednaka nakon obje vrste hranidbe tijekom pohrane mesa na hladnom do 6 dana, ali jače razvijena nakon hranidbe lanom u leđnoj slanini trećeg dana pohrane. Dobiveni rezultati potvrđuju da trajna hranidba tovljenika s relativno niskom razinom lana u obroku može proizvesti svinjsko meso s povećanim udjelom C18:3n-3 i preformiranih C20-22n-3, s nutritivno usklađenim n-6/n-3 omjerom bez negativnih učinaka na tovne performanse i kakvoću trupova i mesa.

Ključne riječi: svinjetina, leđna slanina, n-3 masne kiseline, oksidacija lipida, lan