Journal homepage: http://www.ifrj.upm.edu.my

FOOD RESEARCH

Effects of the dietary inclusion of linseed oil and grape pomace on weight gain, carcass characteristics, and meat quality of swine

^{1*}Trombetta, F., ^{1,3}Fruet, A. P. B., ¹Stefanello, F. S., ¹Fonseca, P. A. F., ²Souza, A. N. M. De, ²Tonetto, C. J., ²Rosado Júnior, A. G. and ¹Nörnberg, J. L.

¹Department of Food Science and Technology, Federal University of Santa Maria, Av. Roraima 1000, CEP 97105-900, Santa Maria, RS, Brazil

²Farroupilha Federal Institute, Rua Vinte de Setembro 2616, CEP 97420-000, São Vicente do Sul,

RS, Brazil

³Agriculture Department of Rio Grande do Sul State, Av. João de Césaro, n.434, CEP 99070-140, Passo Fundo, RS, Brazil

Article history

<u>Abstract</u>

Received: 8 May, 2019 Received in revised form: 4 September, 2019 Accepted: 24 September, 2019

Keywords

Fatty acids Antioxidant Omega-3 Finishing diet Meat characteristics The objective of the present work was to evaluate the effect of diet with the inclusion of linseed oil (*n*-3 fatty acids source) and grape pomace (antioxidant source) on weight gain, carcass characteristics, and meat quality of swine. During 90 days, crossbred swine (n = 44) were fed with four different finishing diets: control diet based on soybean meal and corn (CONT); diet with addition of 3.0% linseed oil (LO); diet with 3.0% linseed oil and 3.5% ensiled grape pomace in dry matter (3.5% EGP); and diet with 3.0% linseed oil and 7.0% ensiled grape pomace in dry matter (7.0% EGP). The finishing diets did not alter daily weight gain although swine fed with LO diet presented thicker backfat when compared to those fed with CONT diet. LO diet significantly increased *n*-3 fatty acid concentration and reduced *n*-6 / *n*-3 ratio when compared to CONT diet. Lipid profile alteration did not significantly affect sensory parameters. The inclusion of EGP (3.5% EGP and 7.0% EGP) increased fibrousness as compared to CONT and LO diets. The inclusion of LO and EGP in finishing diet of swine did not affect weight gain, but generated final product with higher nutritional value (increased polyunsaturated fatty acids / saturated fatty acids (PUFA / SFA) ratio and reduced *n*-6 / *n*-3 ratio) as compared to CONT diet. The sensory parameters of the inclusion diets were similar to those found in CONT diet.

© All Rights Reserved

Introduction

Several bodily functions are regulated by n-3 polyunsaturated fatty acids (PUFA), and these PUFA can also prevent cardiovascular diseases (Marchioli and Levantesi, 2013), cancers (Ferguson, 2010), and metabolic disorders (Julibert *et al.*, 2019). A commercially available source that allows the deposition of n-3 in meat is linseed oil which contains 60% of C18:3n-3 (Soto-Cerda *et al.*, 2014; Kajla *et al.*, 2015). Pork can be an important source of n-3 PUFA if the swine are fed with diets enriched with them; this is due to a large amount of ingested n-3 PUFA will be deposited in tissues, and this is a convenient way to increase the nutritional values of pork and pork products for human consumption (Corino *et al.*, 2014).

In addition to increasing n-3 PUFA deposition and to reduce SFA levels, studies have also confirmed that a high dietary SFA content contributes to higher cholesterol levels leading to increased cardiovascular diseases (Mahan *et al.*, 2011). The main food source that is rich in n-3 PUFA and has an appropriate PUFA / SFA ratio is fish meat and its derivatives; however, this is a product not readily available to large part of the population. Furthermore, pork is not only more affordable but also the most widely consumed meat worldwide. Using an appropriate finishing diet, it is possible to produce pork enriched with n-3 PUFA so that it becomes similar to fish in terms of nutritional value.

The desirable increase in the deposition of PUFA in pork has been associated with increased lipid peroxidation (Contini *et al.*, 2014). The use

of antioxidants is a strategy to neutralise this effect and they can be added directly to the product or fed through diet. Because of the increasing demand for the use of natural over synthetic compounds, and due to increased attention to sustainability of agricultural practices, grape pomace could be incorporated into the animal diet as a feed rich in natural antioxidants and abundant by-product of wine making industry; which represents 20 to 30% of the original grape weight used in the vinification (Dwyer *et al.*, 2014). Grape pomace is also considered as an antioxidant agent due to the phenolic compounds present in it (Soto *et al.*, 2015).

Therefore, the aim of the present work was to evaluate the effects of the dietary inclusion of linseed oil (as a source of n-3 PUFA) and grape pomace (as a source of natural antioxidants) in the weight gain, carcass characteristics, and meat quality of swine.

Materials and methods

Animals, diets, and management

The present work was approved by the Ethics Committee from Farroupilha Federal Institute, Brazil, under the protocol number 10.662.072/0001-58.

A total of 44 swine, aged \pm 180 days, and crossbred from F1 line dams (50% Large White \times 50% Landrace) and Embrapa MS115 line boars, with a mean body weight (BW) of 48.6 kg, were selected and randomly distributed in four batches of 11 swine each (five castrated males and six females). The adaptation period was 14 days to an average of 86 days experimental period. Each batch was fed with different diets: control (CONT), 3.0% linseed oil (LO), 3.0% linseed oil plus 3.5% ensiled grape pomace in dry matter (DM) (3.5% EGP), and 3.0% linseed oil plus 7.0% ensiled grape pomace in DM (7.0% EGP). The level of linseed oil used resulted from the positive changes in fatty acid profile observed in the previous study by Juárez et al. (2010) and Jiang et al. (2017); while GPE incorporation in the regular feed of swine is safe for normal growth, without lowering the productive parameters. Control comprised of corn, soybean meal, mineral and vitamin premix in order to meet the nutritional requirements of the animals (Rostagno et al., 2005). The linseed oil was included directly in the mixer during the concentrate preparation process. All diets were isoproteic, with varying ether extract and carbohydrates contents as shown in Table 1.

Grape pomace (GP) was obtained from rural workers in Jaguari city, Southern Brazil, from red grapes. The grapes (40 kg) were ensiled (EGP) in nylon bags (Sinuelo, Brazil), and packed on the same day as they were pressed in the winery. Upon delivery, the EGP was passed through a stainless steel mesh with 8 mm aperture to reduce the agglomerate size and obtain a homogenous mix with other ingredients. The average composition of GP in dry matter (DM) was: 11.53 g/100 g of crude protein, 3.97 g/100 g of ash, 48.68 g/100 g of neutral detergent fibre, 5.9 g/100 g of ether extract, 29.92 g/100 g of non-fibrous carbohydrates, and 62.95 mg/100 g of phenolic compounds.

Swine of each dietary treatment groups were kept together in concrete pans, with a water channel, a drinking fountain type byte ball (2/pan) and linear troughs (30 cm/swine) with a density of 1.2 m2 per swine. The feed intake was recorded daily, and swine were weighed weekly in order to obtain the average daily gain (ADG). The feed intake was 2.53, 2.49, 2.51, and 2.50% of body weight (BW) for CONT, LO, 3.5% EGP, and 7.0% EGP, respectively; and feed conversion of swine of CONT, LO, 3.5% EGP, and 2.99, 3.02, 3.09, and 2.91, respectively.

When swine reached the slaughter weight of 120 kg, they were submitted to solid fasting for approximately 12 h. Prior to slaughtering, swine were stunned by an electric current of three amps, sufficient to cause an epileptic state preventing brain metabolic activity, and slaughtered immediately after that.

Carcass characteristics

During the slaughter procedure, hot carcass weight (HCW) was recorded. The pH was measured at 45 min post-mortem in the muscle longissimus thoracis (LT), between 11th and 12th ribs of left halfcarcasses using a digital potentiometer PH-2600 (Icel Manaus, Manaus, Amazônia, Brazil) equipped with a penetration electrode and a temperature probe. The carcasses were then stored in a cold room at 2°C for 24 h after which the cold carcass weight (CCW) were recorded in order to calculate the cold carcass yield $(CCY = CCW \times 100/LV)$ and cooling break (CB = $[(HCW - CCW)/HCW] \times 100)$. After 24 h, pH was also measured again (between 11th and 12th ribs of left half-carcasses). For visual assessments of colour and marbling of the muscle, a cross section was taken between 11th and 12th ribs and after 20 min of exposure to the air for pigment stabilisation. Grades from 1 to 6 were attributed using image standards (1 = very light and 6 = very dark). For the subjective analysis of marbling, image standards were used to attribute grades from 1 to 5 (1 = trace marbling)and 5 = abundant marbling). Both assessments were performed using Pork Quality Standards image tables

from National Pork Producers Council - NPPC-1991 (Aberle *et al.*, 2012).

The loin eye area (LEA) was obtained from the same cross section using a planimeter through the exposure of LT muscle, traced on acetate paper following the ABCS guidelines (ABCS, 1973). Backfat thickness (BFT) was measured at the level of first rib, last rib, last lumbar vertebrae (LLV), and half-carcass cross section between 11th and 12th ribs, using a digital pachymeter. Drip loss was assessed by the collection of 100 g fraction of LT muscle, placed in a reticulate bag, refrigerated at 2 - 4°C for 24 h, and re-weighed. Drip loss was given as the difference between the initial and the final sample weight. Next, LT muscle was chopped in 2.5 cm thick chops, and cut perpendicularly to the muscle. The samples were identified, vacuum-sealed in low gas permeability polyamide packs, and stored at -18°C in the dark until they were shipped to the laboratory for analysis.

Meat quality evaluation

Thirty grams of LT were lyophilised (Terroni, LS3000B, Brazil) under optimal conditions (Carpentier *et al.*, 2007) for chemical composition analysis. Moisture, crude protein, and ash were quantified based on AOAC (1995). The remaining portion of the steak was used to determine the total

Table 1. Ingredients, composition of experimental diets, centesimal composition, fatty acids profile, and phenolic compounds.

	Diet					
	¹ CONT	¹ LO	¹ 3.5% EGP	¹ 7.0% EGP		
Ingredient (g/kg)						
Corn	822	786	754	718		
Soybean meal	144	150	145	139		
Mineral-Vitamin Premix*	34	34	37	39		
Linseed oil	0	30	30	30		
Ensiled grape pomace**	0	0	34	74		
Diet composition (g/100 g of dry matter)					
Moisture	7.09	6.88	9.47	12.06		
Crude protein	18.54	17.98	17.44	17.49		
Ash	6.59	6.39	6.30	6.21		
Neutral detergent fibre	14.58	14.14	15.34	16.53		
Ether extract	5.45	8.29	8.30	8.32		
Non-fibrous carbohydrate	54.84	53.19	52.32	51.45		
Phenolic compounds (mg/100 g)	-	-	2.20	4.41		
Diet fatty acid profile (g/100 g fatty acid	d methyl esters)					
14:0	0.86	0.84	0.81	0.79		
16:0	34.93	34.07	33.14	32.21		
16:1 <i>n</i> -7	0.62	0.60	0.59	0.58		
18:0	7.09	7.04	6.92	6.80		
18:1 <i>n</i> -9	23.95	23.84	23.71	23.59		
18:2 <i>n</i> -6	29.65	29.19	30.14	31.10		
18:3 <i>n</i> -3	1.52	3.04	3.25	3.47		
20:0	0.42	0.41	0.41	0.40		
SFA	43.30	42.36	41.28	40.21		
MUFA	24.57	24.44	24.31	24.17		
PUFA	31.17	32.22	33.39	34.56		
<i>n</i> 6	29.65	29.19	30.14	31.10		
<i>n</i> 3	1.52	3.04	3.25	3.47		

*Minerals (Mg, Mn, Fe, Cu, Zn, Se); Vitamins (A, B1, B2, B6, B12, D, K, Biotin (B3)); Amino acids (lysine, methionine, tryptophan, histidine, isoleucine, leucine, threonine, valine, arginine, phenylalanine). ** 28% DM. ¹CONT = control; LO = 3.0% linseed oil; 3.5% EGP = linseed oil and 3.5% ensiled grape pomace; 7.0% EGP = linseed oil and 7.0% ensiled grape pomace. ²C14:0 = myristic acid; C16:0 = palmitic acid; C16:1n7 = palmitoleic acid; C18:0 = estearic acid; C18:1n9 = oleic acid; C18:2n6 = linoleic acid; C18:3n3 = alpha-linolenic acid; C20:0 = arachidic acid; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n3 = C18:3n3; n6 = C18:2n6.

lipid values (Hara and Radin, 1978) and fatty acid (FA) profile (Christie, 1982). Fatty acid methyl esters (FAME) were quantified using a gas chromatograph (GC) (Agilent, 45,813–01, CA, EUA), equipped with a flame ionisation detector (FID). Analyses were performed using a fused-silica capillary column (0.25 mm \times 60 m, Supelco SPTM - 2362, PA, EUA). Oven temperatures were set from 100°C to 240°C, while injector and detector temperatures were 250°C and 280°C, respectively. Carrier gas used was nitrogen at a flow of 0.6 mL/ min. Fatty acids were identified by comparing retention times to previously known standards (mix 37, linoleic acid methyl ester; cis-7,10,13,16,19 - docosapentaenoic acid methyl ester). Fatty acids were quantified by the incorporation of an internal standard with known concentration (methyl tricosanoic acid - C23:0) in each sample prior to methylation, as well as using a theoretical correction factor and a conversion factor from methyl ester to FA, following the method proposed by Tonial et al. (2014). Cholesterol determination was performed by the enzymatic method with laboratory kits adapted from Saldanha et al. (2004). In order to calculate the water-holding capacity, a sample of 0.5 g was weighed in filter paper and pressed for 5 min using 2.25 kg weight. After compression, samples were weighed again following the method adapted from Osório (1998).

For the calculation of cooking loss (CL), Warner-Bratzler shear force (WBSF), and sensory evaluation, steaks were thawed for 24 h at 4°C and grilled using an electric grill. Steaks were flipped at 35°C and cooked further until the final temperature reached 71°C in the geometric centre. Cooking loss was calculated from steaks used for WBSF using Eq. 1:

Cooking loss $\% = [100 - (grilled weight of the steak / raw weight of the steak)] \times 100)$ (Eq. 1)

For WBSF, steaks were cooled overnight at 4°C and six cores of 1.27 cm in diameter were taken in parallel to the muscle fibre orientation. Shear force and texture profile were evaluated using a texture analyser (Stable Micro Systems, TA.XTplus). V shaped (60°) Warner Bratzler blade was used for shear force evaluation. Test speed was set in 200 mm per minute. Texture profile was assessed by the compression of six 1.5 cm3 cubes, using a 36 mm diameter cylindrical probe at a constant speed of 60 mm/min. Cubes were compressed twice at 80% of the sample thickness. Texture parameters assessed were hardness, adhesiveness, cohesiveness, springiness, fibrousness, shear force, resilience, and chewiness.

Determination of thiobarbituric acid reactive substances (TBARS) was performed following the method described by Raharjo *et al.* (1992). Results were calculated from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) (T9889, Sigma-Aldrich, St. Louis, USA) and were expressed in milligrams of malondialdehyde (MDA) per kg of sample.

Instrumental colour was recorded using the parameters lightness (L*), redness (a*), yellowness (b*), saturation (c*), and hue angle (H*) with a Minolta Chromameter (CM-700D, Minolta Inc., Japan); with a measuring area of 8 mm diameter, calibrated for illuminant A and at 10° standard observer.

Sensory analysis

A total of 11 panellists between 18 and 30 years old of age were trained following AMSA (2015) guidelines. For each session, 16 LT samples were served to the panellists (two steaks per swine, two swine per treatment, four treatments per session). After removal of subcutaneous fat, steaks were cooked until an internal temperature of 71°C was reached, and 44 cube-shaped samples (22 from each steak, dimensions: $2.54 \times 1.27 \times 1.27$ cm) were served to the panellists. Samples were individually served to each panel members, under red light to avoid visual differences. Salt-free crackers and water were available for ingestion by the panel members between samples to cleanse their palate. Panel members evaluated juiciness from 1 = extremely dry to 7 =extremely juicy; initial and overall tenderness from 1 = extremely tough to 7 = extremely tender; pork flavour from 1 = extremely bland to 7 = extremely intense. Off-flavour intensity was also assessed using an eight-point scale (0 = none, 1 = extremely blandto 8 = extremely intense). Four sessions were carried out.

Statistical analysis

Data was analysed in a completely randomised design, while for the sensory analysis, a panellist was used as block to improve precision (Gacula, 1993). Analysis was conducted using the GLM SAS procedure (Version 9.3, Cary, NC). Normality of residuals and homogeneity were assessed by Shapiro-Wilk and Levene test (p > 0.05). While ANOVA resulted in a significant p-value (p < 0.05), means were compared using Tukey test (p < 0.05).

Results and discussion

Weight gain and carcass characteristics

Effects of the finishing diets in weight gain and carcass characteristics are presented in Table 2. The inclusion of LO and EGP in the concentration reported did not affect the ADG, animal age at slaughtering, HCY, initial pH, pH at 24 h, drip loss, visual colour or marbling, LEA, BFT at first rib, BFT at last rib, and BFT at LLV when compared to the CONT diet. This is in agreement with studies by Matthews et al. (2000), Corino et al. (2008), and Okrouhlá et al. (2013), in which effects on animal weight gain characteristics due to linseed oil addition to their diets was not found. On the other hand, lumbar backfat thickness differed between treatments of LO and CONT in the present work. Based on similarity of feed intake and feed conversion between diets, the backfat thickness possibly increased due to increased energy availability in LO diets when compared to CONT. Similar results were showed by Cline et al. (2016). Changes in carcass composition, which shifted between lean tissue and fat, can be attributed to the degree of maturity of animals. When animals approach maturity, there is a significant decrease in lean tissue development, which can decline to zero when the animals reaches adult body size. In this situation, as long as there is no energy limitation, swine can continue depositing fat.

Meat quality

There was no effect in regard to diets in the centesimal composition, cholesterol, water-holding

capacity (WHC), cooking loss (CL), and in the texture profile analysis (TPA) as shown in Table 3.

TBARS values were higher in the meat of swine fed with 7.0% EGP than LO and CONT. Lipid oxidation levels quantified in the present work were low, ranging from 0.008 to 0.056 mg MDA/kg of muscle. This could be due to the initial phase of the oxidative process and different results could be observed if meat oxidative stability was assessed over a longer period of refrigeration in aerobic conditions. O'Grady *et al.* (2008), in a study using grape seed extract, evaluated the oxidative stability for 16 days storage at 4°C, and observed a reduction in lipid oxidation at days 8 and 12 in the group whose feed contained 700 mg/kg of grape seed extract.

Gray and Pearson (1987) suggested 1 mg of MDA/kg in the muscle as a threshold for the organoleptic detection of rancidity. Thus, the possible EGP antioxidant effect may have been limited in the initial storage period which is in agreement with reports from Bertol et al. (2017) that did not get a protective effect on the meat lipids by the use of GP in swine finishing diet. Possibly, longer periods of GP inclusion in the diet or shelf-life analysis can detect antioxidant effect on pork. According to O'Grady et al. (2008), hydrophilic and chemical nature of compounds present in GP can influence metabolism, bioavailability, absorption, and metabolite excretion from animals. Thus, antioxidant effect, measured by TBARS production, was not detected in the present work.

Meat colour is the main feature affecting consumers' buying decision as redness is associated

	0 0			1		
	¹ CONT	¹ LO	¹ 3.5% EGP	¹ 7.0% EGP	<i>p</i> -value	SME
² ADG (kg/day)	0.85	0.84	0.81	0.86	0.6539	0.014
Animal age at slaughter (days)	180.90	181.81	184.09	175.45	0.2298	1.50
² HCY (%)	82.70	84.89	84.45	81.59	0.3800	0.44
Initial pH	6.11	5.91	5.94	6.01	0.0503	0.028
pH 24 h	5.34	5.30	5.28	5.30	0.2415	0.005
Drip loss (%)	7.42	10.66	7.9	8.6	0.2364	0.65
Subjective colour	3.36	3.27	2.90	3.54	0.1940	0.47
Subjective marbling	2.72	2.81	3.18	2.54	0.2561	0.55
² LEA	41.82	45.48	49.11	46.68	0.5120	1.55
² BFT 1 st rib (mm)	3.71	4.18	4.10	3.75	0.1605	0.08
² BFT 13 th rib (mm)	2.00	2.38	2.17	2.34	0.07204	0.05
² BFT LLV (mm)	3.26	3.43	3.68	3.58	0.1521	0.06
² BFT 11 th - 12 th (mm)	1.57 ^b	1.96ª	1.62 ^{ab}	1.69 ^{ab}	0.0495	0.05

Table 2. Weight gain and carcass characteristics of swine feed experimental diets.

Means followed by different letters on the same line significantly differ among themselves (p < 0.05). SME = standard mean error. ¹CONT = control; LO = 3.0% linseed oil; 3.5% EGP = linseed oil and 3.5% ensiled grape pomace; 7.0% EGP = linseed oil and 7.0% ensiled grape pomace. ²ADG = average daily gain; HCY = hot carcass yield, LEA = loin eye area, BFT 1st rib = backfat thickness at the level of the first rib; BFT 13th rib = backfat thickness at the level of the last rib; BFT LLV= backfat thickness at the level of the last lumbar vertebrae; BFT 11th - 12th = backfat thickness at the level of the 11th and 12th ribs.

thoracis muscle of swine led with the experimental diets.							
	¹ CONT	¹ LO	¹ 3.5% EGP	¹ 7.0% EGP	<i>p</i> -value	SME	
Cholesterol (mg/100 g)	64.94	66.69	64.04	65.89	0.5071	0.64	
WHC (g 100/ g)	64.30	63.06	65.46	65.43	0.5514	0.67	
CL	33.65	37.07	36.15	35.30	0.1501	0.50	
Lipid (g/100 g)	7.39	7.16	7.66	7.05	0.1964	0.11	
Moisture (g/100 g)	70.85	71.60	71.55	72.13	0.1269	0.18	
Ash (g/100 g)	1.11	1.11	1.12	1.15	0.8795	0.02	
Protein (g/100 g)	21.51	21.35	20.86	20.96	0.4022	0.15	
ΓBARS	0.008c	0.023cb	0.035ab	0.053a	< .0001	0.003	
Colour							
Lightness (L*)	50.48	47.01	53.68	53.80	0.4341	0.44	
Redness (a*)	12.10	10.80	11.36	12.58	0.5257	0.42	
(b*)	12.04	11.30	13.06	13.51	0.3182	0.44	
Saturation (C*)	141.52	162.57	159.19	171.99	0.5313	2.86	
Hue angle (H*)	46.02	46.01	46.93	47.04	0.1084	0.20	
Texture							
Warner-Bratzler shear force (N)	5.28	5.30	5.77	4.97	0.6012	0.19	
Hardness (N)	193.58	221.89	200.36	208.79	0.3012	5.27	
Cohesiveness	0.41	0.41	0.39	0.40	0.5124	0.0052	
Adhesiveness (N.cm)	-0.03	-0.04	-0.01	-0.00	0.0630	0.0062	
pringiness	1.34	1.27	1.30	1.42	0.1822	0.022	
Chewiness (N)	110.50	115.81	102.07	120.50	0.4503	3.83	
Resilience	0.18	0.19	0.17	0.17	0.5929	0.0038	

 Table 3. Cholesterol, water-holding capacity, centesimal composition, and meat quality assessment in longissimus thoracis muscle of swine fed with the experimental diets.

Means followed by different letters on the same line significantly differ among themselves (p < 0.05); SME = standard mean error. ¹CONT = control; LO = 3.0% linseed oil; 3.5% EGP = linseed oil and 3.5% ensiled grape pomace; 7.0% EGP = linseed oil and 7.0% ensiled grape pomace. ²WHC = water-holding capacity; CL = cooking loss.

with freshness. With regard to colour parameters presented in Table 3, there was no statistically difference among diets for L*, a*, b*, c* (saturation), and hue angle H* values. Bertol *et al.* (2017) documented an increased intensity of a* when feeding swine with 6 to 10% GP, levels that are higher than the ones used in the present work, showing that GP reduced myoglobin oxidation, even though lipid oxidation values were not altered.

Fatty acids profile

Reducing the n-6 / n-3 ratio is a major challenge for the development of healthier meat products. Therefore, the United Nations Food and Agriculture Organization (FAO / WHO) recommends balancing n-6 and n-3 FA intake and Wood *et al.* (2004) recommend that the n-6 / n-3 ratio in meat is less than 4, thus reducing the risk of health problems. Unbalanced PUFA levels may favour the onset of many chronic diseases, and are of great concern to human health, as high intakes of n-6 and n-3 are related to the increased incidence of inflammatory and autoimmune diseases, various types of cancer and cardiovascular diseases (Lee *et al.*, 2012). This is significant because pork with modified FA profiles is of nutritional advantage and health benefits for average pork consumers (Corino *et al.*, 2014).

Table 4 shows that swine fed with diets containing LO and EGP presented an increase in n-3 FA and PUFA levels when compared to CONT. Accordingly, PUFA / SFA ratio increased and n-6 / n-3 ratio decreased, indicating that a final product with improved quality is obtained with diets LO, 3.5% EGP and 7.0% EGP. Once n-6 / n-3 ratio was in the recommended range (lower than 4), it conferred healthier characteristics to the food product in order to fulfil the demands from consumers concerned with their health due to the low SFA content and healthy n-6 / n-3 ratio (Rubilar *et al.*, 2012).

Increased deposition of *n*-3 in meat was also reported by other studies using LO supplementation in swine diet (Kouba *et al.*, 2003; Corino *et al.*, 2008; Juárez *et al.*, 2011; Turner *et al.*, 2014; Jiang *et al.*, 2017; De Tonnac *et al.*, 2017). This increase occurs

Table 4. Fatty acids	profile, quantified	in mg/g of FAME, fr	om swine fed with the ex	perimental diets.

	¹ CONT	¹ LO	¹ 3.5% EGP	¹ 7.0% EGP	<i>p</i> -value	SME
² 14:0	14.03ª	12.41 ^b	12.32ь	12.18 ^b	0.0057	0.22
² 16:0	228.42ª	210.87 ^b	209.79ь	208.84 ^b	0.0024	2.30
² 16:1 <i>n</i> 7	26.60	23.55	23.53	23.14	0.4352	0.52
² 18:0	124.95	116.39	115.51	115.60	0.0439	1.39
² 18:1 <i>n</i> 9	341.75	337.65	323.54	318.67	0.5170	3.39
² 18:2 <i>n</i> 6	69.37 ^ь	71.05 ^b	79.78 ^{ab}	83.84ª	0.0110	1.17
² 18:3 <i>n</i> 3	3.24 ^b	28.24ª	28.44ª	28.78ª	< .0001	1.8
² 20:0	2.65	2.12	2.11	2.09	0.5733	0.16
² 20:1 <i>n</i> 9	7.08 ^a	5.80 ^b	5.70 ^b	5.69 ^b	< .0001	0.13
² 20:4 <i>n</i> 6	11.38 ^a	6.51 ^b	6.52 ^b	6.57 ^b	< .0001	0.42
² 20:3 <i>n</i> 3	0.72 ^b	4.90ª	4.80 ^a	4.82ª	< .0001	0.29
² 20:5 <i>n</i> 3	0.39ь	2.71ª	2.60ª	2.66ª	< .0001	0.17
² 22:4 <i>n</i> 6	2.25 ^a	0.74 ^b	0.67 ^b	0.66 ^b	< .0001	0.12
²SFA	370.05ª	341.79 ^ь	339.73 ^b	338.71 ^b	0.0028	3.60
² MUFA	375.43	367.00	352.77	347.50	0.0830	3.33
² PUFA	87.36 ^b	112.78ª	122.81ª	126.90ª	< .0001	3.30
² PUFA/SFA	0.23 ^b	0.33ª	0.36 ^a	0.37ª	< .0001	0.12
² <i>n</i> 6	83.00	78.30	86.97	91.07	0.2877	2.30
²n3	4.35 ^b	35.85ª	35.84 ^a	35.82ª	< .0001	2.08
² <i>n</i> 6/ <i>n</i> 3	19.82ª	2.18 ^b	2.42 ^b	2.53 ^b	< .0001	1.10

Means followed by different letters on the same line significantly differ among themselves (p < 0.05); SME = standard mean error. 'CONT = control; LO = 3.0% linseed oil; 3.5% EGP = linseed oil and 3.5% ensiled grape pomace; 7.0% EGP = linseed oil and 7.0% ensiled grape pomace. ²C14:0 = myristic acid; C16:0 = palmitic acid; C16:1*n*7 = palmitoleic acid; C18:0 = estearic acid; C18:1*n*9 = oleic acid; C18:2*n*6 = linoleic acid; C18:3*n*3 = alpha-linolenic acid; C20:0 = arachidic acid; C20:1*n*9 = eicosenoic acid; C20:4*n*6 = arachidonic acid; C20:3*n*3 = eicosatrienoic acid; C20:5*n*3 = eicosapentaenoic acid (EPA); C22:4*n*6 = adrenic acid; SFA = saturated fatty acids (C18:2*n*6, C18:3*n*3, C20:3*n*3, C20:5*n*3, C22:4*n*6); *n*6 = C18:2*n*6, C20:4*n*6, C22:4*n*6; *n*3 = C18:3*n*3, C20:5*n*3.

Table 5. Sensory evaluation of longissimus thoracis muscle from swine fed with the experimental diets.

	¹ CONT	¹ LO	¹ 3.5% EGP	¹ 7.0% EGP	<i>p</i> -value	SME
² Juiciness	5.33	5.25	5.08	5.00	0.4844	0.071
² Softness	5.25	5.42	5.04	5.08	0.4884	0.071
² Overall	4.29	4.33	4.21	4.33	0.9654	0.071
² Flavour	0.29	0.25	0.54	0.42	0.6930	0.071
³ Off-flavour	6.50	6.50	6.50	6.50	-	0.071

Means followed by different letters on the same line significantly differ among themselves (p < 0.05); SME = standard mean error. ¹CONT = control; LO = 3.0% linseed oil; 3.5% EGP = linseed oil and 3.5% ensiled grape pomace; 7.0% EGP = linseed oil and 7.0% ensiled grape pomace. 2seven-point scale: 1 = extremely dry, tough and mild to 7 = extremely juicy and intense. 3eight-point scale: 0 = none, 1 = extremely slight to 8 = extremely intense.

due to the lipid profile of linseed oil, characterised by its high PUFA content (45 to 55% of total FA), especially *n*-3, moderate MUFA and low SFA.

Sensory analysis results are presented in Table 5 which shows no statistically difference in sensory characteristics in pork obtained from swine fed with different diets. This agrees with previous studies (Matthews *et al.*, 2000; Kouba *et al.*, 2003). Although there was a remarkable and desirable modification in the lipid profile of swine fed with LO and EGP enriched diets, with increased *n*-3 and PUFA levels, an off-flavour difference was not detected among the different treatments.

Conclusion

The inclusion of LO has been demonstrated to reduce the deposition of SFA and increase *n*-3 PUFA levels, mostly C18:3*n*-3, C20:3n-3 and C20:5*n*-3 in LT muscle, thus reducing the *n*-6 / *n*-3 ratio to < 4:1. The inclusion of LO did not alter the sensory perception of pork, which demonstrates that it is possible to obtain pork enriched with *n*-3 and offer consumers a product with improved functional and / or nutraceutical properties. Even though the present work could not evidence the antioxidant effect of EGP inclusion in swine diet on the meat lipid oxidation at

the levels used, this by-product can be used in the finishing of swine with no detrimental effects on the weight gain and meat quality. Possibly, the protective effects of lipid oxidation were not verified due to the low levels of EGP inclusion in the present work. Therefore, it is suggested to continue the studies with higher inclusion levels, as well as evaluations of the oxidative stability of the lipid meat over time.

Acknowledgement

The authors would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil) for the scholarship provided by the social demand program (DS, Brazil) for the first author, and the National Council for Scientific and Technological Development (CNPq, Brazil) for awarding the Research Grant to the final author.

References

- Aberle, E. D., Forrest, J. C., Gerrard, D. E. and Mills, E. W. 2012. Principles of meat processing. In Principles of Meat Science (5th ed), p. 117-153. United States: Kendall Hunt Publishing.
- American Meat Science Association (AMSA). 2015. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of meat. Retrieved on April 6, 2019 from website: http://www. meatscience.org/docs/default-source/publicationsresources/amsa-sensory-and-tenderness-evaluationguidelines/research-guide/2015-amsa-sensoryguidelines-1-0.pdf?sfvrsn=6
- Association of Official Analytical Chemists (AOAC). 1995. Official methods of analysis of AOAC International. (16th ed). United States: AOAC.
- Bertol, T. M., Ludke, J. V., Campos, R. M. L., Kawski, V. L., Cunha Junior, A. and Figueiredo, E. A. P. 2017. Inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. Ciência Rural 47(4): article ID e20150358.
- Brazilian Association of Pig Breeders (ABCS). 1973. Brazilian method of carcass classification. Estrela, Brazil: ABCS.
- Carpentier, S. C., Dens, K., Van den Houwe, I., Swennen, R. and Panis, B. 2007. Lyophilization, a practical way to store and transport tissues prior to protein extraction for 2-DE analysis? Proteomics 7(Suppl. 1): 64-69.
- Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. Journal of Lipid Research 23(7): 1072-1075.
- Cline, P. M., Tsai, T. C., Stelzleni, A. M., Dove, C. R. and Azain, M. 2016. Interaction of dietary energy and protein on growth performance, carcass characteristics and digestibility in finishing barrows when fed at a constant digestible lysine to metabolizable energy ratio. Livestock Science 184: 1-6.

- Contini, C., Álvarez, R., O'Sullivan, M., Dowling, D. P., Gargan, S. Ó. and Monahan, F. J. 2014. Effect of an active packaging with citrus extract on lipid oxidation and sensory quality of cooked turkey meat. Meat Science 96(3): 1171-1176.
- Corino, C., Musella, M. and Mourot, J. 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 liveweight. Journal of Animal Science 86(8): 1850-1860.
- Corino, C., Rossi, R., Cannata, S. and Ratti, S. 2014. Effect of dietary linseed on the nutritional value and quality of pork and pork products: systematic review and meta-analysis. Meat Science 98(4): 679-688.
- De Tonnac, A., Karim-Luisset, S. and Mourot, J. 2017. Effect of different dietary linseed sources on fatty acid composition in pig tissues. Livestock Science 203: 124-131.
- Dwyer, K., Hosseinian, F. and Rod, M. 2014. The market potential of grape waste alternatives. Journal Food Research 3(2): 91-106.
- Ferguson, L. R. 2010. Meat and cancer. Meat Science 84(2): 308-313.
- Gacula, M. C. 1993. Design and analysis of sensory optimization. United States: Food and Nutrition Press.
- Gray, J. I. and Pearson, A. 1987. Rancidity and warmedover flavour. In Pearson, A. M. and Dutson, T. R. (eds). Advances in Meat Research Volume 3: Restructured Meat and Poultry Products, p. 221-269. New York: Van Nostrand Reinhold Co.
- Hara, A. and Radin, N. S. 1978. Lipid extraction of tissues of low toxicity solvent. Analytical Biochemistry 90(1): 420-426.
- Jiang, J., Tang, X., Xue, Y., Lin, G. and Xiong, Y. L. 2017. Dietary linseed oil supplemented with organic selenium improved the fatty acid nutritional profile, muscular selenium deposition, water retention, and tenderness of fresh pork. Meat Science 131: 99-106.
- Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T. and Beaulieu, A. D. 2011. Increasing omega-3 levels through dietary coextruded flaxseed supplementation negatively affects pork palatability. Food Chemistry 126(4): 1716-1723.
- Juárez, M., Dugan, M. E. R., Aldai, N., Aalhus, J. L., Patience, J. F., Zijlstra, R. T. and Beaulieu, A. D. 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 Science 84(3): 578-584.
- Julibert, A., Bibiloni, M. D. M., and Tur, J. A. 2019. Dietary fat intake and metabolic syndrome in adults: a systematic review. Nutrition, Metabolism and Cardiovascular Diseases 29(9): 887-905.
- Kajla, P., Sharma, A. and Sood, D. R. 2015. Flaxseed a potential functional food source. Journal of Food Science and Technology 52(4): 1857-1871.
- Kouba, M., Enser, M., Whittington, F. M., Nute, G. R. and Wood, J. D. 2003. Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. Journal of Animal

Science 81(8): 1967-1979.

- Lee, S. P., Dart, A. M., Walker, K. Z., O'dea, K., Chin, J. P. and Skilton, M. R. 2012. Effect of altering dietary *n*-6:*n*-3 PUFA ratio on cardiovascular risk measures in patients treated with statins: a pilot study. The British Journal of Nutrition 108(7): 1280-1285.
- Mahan, K. L, Escott-Stump, S. and Raymond, J. L. 2011. Krause's food and the nutrition care processes. (13th ed). United States: Saunders.
- Marchioli, R. and Levantesi, G. 2013. N-3 PUFAs in cardiovascular disease. International Journal of Cardiology 170(2 Suppl. 1): S33-S38.
- Matthews, K. R., Homer, D. B., Thies, F. and Calder, P. C. 2000. Effect of whole linseed (Linum usitatissimum) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. The British Journal of Nutrition 83(6): 637-643.
- O'Grady, M. N., Carpenter, R., Lynch, P. B., O'Brien, N. M. and Kerry, J. P. 2008. Addition of grape seed extract and bearberry to porcine diets: Influence on quality attributes of raw and cooked pork. Meat Science 78(4): 438-446.
- Okrouhlá, M., Stupka, R., Čítek, J., Šprysl, M. and Brzobohatý, L. 2013. Effect of dietary linseed supplementation on the performance, meat quality, and fatty acid profile of pigs. Czech Journal of Animal Science 58(6): 279-288.
- Osório, J. C. S. 1998. Métodos para avaliação da produção de carne ovina: "in vivo" na carcaça e na carne. Brazil: Universidade Federal de Pelotas (UFPel).
- Raharjo, S., Sofos, J. N. and Schmidt, G. R. 1992. Improved speed, specificity and limit of determination of an aqueous acid extraction thiobarbituric acid-C18 method for measuring lipid peroxidation in beef. Journal of Agricultural and Food Chemistry 40(11): 2182-2185.
- Rostagno, H. S. 2005. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. (2nd ed). Brazil: Federal University of Viçosa.
- Rubilar, M., Morales, E., Contreras, K., Ceballos, C., Acevedo, F., Villarroel, M. and Shene, C. 2012. Development of a soup powder enriched with microencapsulated linseed oil as a source of omega-3 fatty acids. European Journal of Lipid Science and Technology 114(4): 423-433.
- Saldanha, T., Mazalli, M. R. and Bragagnolo, N. 2004. Comparative evaluation between two methods for determining cholesterol in meat and milk. Food Science and Technology 24(1): 109-113.
- Soto, M. L., Falqué, E. and Domínguez, H. 2015. Relevance of natural phenolics from grape and derivative products in the formulation of cosmetics. Cosmetics 2(3): 259-276.
- Soto-Cerda, B. J., Duguid, S., Booker, H., Rowland, G., Diederichsen, A. and Cloutier, S., 2014. Association mapping of seed quality traits using the Canadian flax (Linum usitatissimum L.) core collection. Theoretical and Applied Genetics 127(4): 881-896.

- Tonial, I. B., Oliveira, D. F., Coelho, A. R., Matsushita, M., Coró, F. A. C., Souza, N. E. and Visentainer, J. V. 2014. Quantification of essential fatty acids and assessment of the nutritional quality indexes of lipids in tilapia alevins and juvenile tilapia fish (Oreochromis niloticus). Journal of Food Research 3(3): 105-114.
- Turner T. D., Mapiye, C., Aalhus, J. L., Beaulieu, A. D., Patience J. F., Zijlstra R. T. and Dugan M. E. R. 2014. Flaxseed fed pork: *n*-3 fatty acid enrichment and contribution to dietary recommendations. Meat Science 96(1): 541-547.
- Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., ... and Enser, M. 2004. Effects of fatty acids on meat quality: a review. Meat Science 66(1): 21-32.