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Effects of high linolenic acid diet supplemented with synthetic or natural antioxidant mix on live performance, carcass traits, meat quality and fatty acid composition of *Longissimus thoracis et lumborum* muscle of medium-heavy pigs

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PAPER

Abstract

We studied the effect of a high linolenic acid diet supplementation with synthetic (vitamin E + selenium) or vegetal mix rich in natural antioxidants (grape skin + oregano) on live performances, carcass and meat quality, fatty acid composition and oxidative stability of intramuscular lipids of *Longissimus thoracis et lumborum* muscle in medium-heavy pigs. Neither carcass traits nor chemical proximate composition of meat was affected by dietary treatments. Linseed dietary inclusion reduced the n-6:n-3 polyunsaturated fatty acids ratio and increased long-chain n-3 precursor, fundamental for human health. Our results offer new opportunities to use products more acceptable by consumers and are more eco-friendly.

Keywords: extruded linseed, fatty acids profile, grape skin extract, oregano extract, oxidative stability of pork, vitamin E

Introduction

Polyunsaturated fatty acids (PUFA), including n-6 and n-3 groups, are essential for physiological functioning and health of humans and domestic animals (Delgado-Lista *et al.*, 2012). Western diets are deficient in n-3 PUFA and contain excessive amounts of n-6 PUFA, resulting in a high n-6:n-3 ratio ranging from 10:1 to 20:1, while an optimal recommended ratio is 1:1–4:1. High values of ratio can be the cause of various diseases (Simopoulos, 2002, 2010; Leslie *et al.*, 2015). Many studies have shown that n-3, mainly eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) fatty acids (FA), have an anti-inflammatory effect and play a beneficial role in a number of human diseases, including autoimmune diseases, diabetes, tumours, Alzheimer's disease and stroke (Boston *et al.*, 2004; Fritsche, 2006; Wall *et al.*, 2010).

α-Linolenic acid (ALA, 18:3 n-3) is a precursor of n-3 PUFA group; EPA, docosapentaenoic (DPA, 22:5 n-3) and DHA fatty acids are synthesized from ALA through consecutive elongation and desaturation.

In general, animal fats are lacking in n-3 PUFA, while contain n-6 PUFA abundantly. Hence, pork products provide too much of n-6 while lacking in n-3 PUFA (Wood *et al.*, 2008; Kouba and Mourot, 2011). Owing to a high content of saturated fatty acids (SFA) and an

unfavourable n-6:n-3 PUFA ratio (Liu and Kim, 2018), pork consumption has been associated with an increased risk of chronic diseases (Egeberg *et al.*, 2013; Klurfeld, 2015).

However, genetic factors (Cameron *et al.*, 2000; Piedrafita *et al.*, 2001; Wood *et al.*, 2008), sex, age, live weight at slaughter (Lebret and Mourot, 1998; Lo Fiego *et al.*, 2005b; Minelli *et al.*, 2019) and feeding strategies (Lo Fiego *et al.*, 2005a) can influence deposition of lipids in pig tissues and their fatty acid composition. It is widely accepted that nutrition is the main factor influencing deposition of lipid and fatty acid in monogastric animals. Dietary FA can significantly modify the fatty acid profile of adipose tissues in pigs. In fact, many studies have confirmed the enrichment of pig tissues with n-3 PUFA by incorporating linseed in pig diets (Kouba *et al.*, 2003; Corino *et al.*, 2008; Minelli *et al.*, 2020) or its derivatives rich in ALA (Guillevic *et al.*, 2009; Musella *et al.*, 2009; Corino *et al.*, 2014).

However, increasing PUFA and lowering SFA contents raises the susceptibility of meat to lipid oxidation, leading to undesirable effects such as deterioration of its sensorial quality and nutritional value (Rivas-Cañedo *et al.*, 2013; Chamorro *et al.*, 2015). To counteract this effect, dietary addition of antioxidants, such as vitamin E and selenium, is widely used in swine feeding. Supranutritional levels of vitamin E may improve oxidative and colour stability of meat during storage (De la Fuente *et al.*, 2009; Kasapidou *et al.*, 2012; Muíño *et al.*, 2014). Moreover, the combination of vitamin E and selenium can build a complex antioxidant system capable of protecting against free radicals and lipid oxidation products (Surai and Fisinin, 2015). However, now consumers feel more reasonable the use of natural antioxidant products in animal feeding (Gladine *et al.*, 2007; Brenes *et al.*, 2016). Among these, oregano extract has shown a considerable antioxidant effect (Botsoglou *et al.*, 2003) as well as antimicrobial and anti-inflammatory activity (Cheng *et al.*, 2017). Oregano antioxidant action is attributed to the presence of different phenolic active compounds such as thymol and carvacrol (Tuttolomondo *et al.*, 2013).

Another very rich natural source of antioxidants is represented by various by-products of winery, such as grape skin and seeds, that are rich in polyphenolic compounds characterized by a high antioxidant activity (Selani *et al.*, 2011).

Furthermore, the wine industry produces a large amount of residues in a short period of the year whose disposal is expensive and involves problems related to pollution (Bustamante *et al.*, 2008).

The inclusion of these by-products as an alternative to synthetic antioxidants could be an interesting nutritional and environment-friendly strategy.

The aim of this research was to study the effects of inclusion of linseed in the growing–finishing diet of medium-heavy pigs and of the supplementation with supranutritional levels of a synthetic antioxidant complex (vitamin E and selenium) or vegetal mix rich in natural antioxidants (grape skin and oregano) on live performance, carcass and meat quality, and on fatty acid composition and oxidative stability of intramuscular lipids of *Longissimus thoracis et lumborum* (LTL) muscle.

MATERIALS AND METHODS

Ethics approval

All the experimental procedures performed in this study were in accordance with the Italian legislation and did not require special animal care authorizations, that is the decision of the Animal Welfare Committee of Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA; 14 September 2016), according to the Italian Legislative Decree of 4 March 2014 n. 26 art. 2, point F.

Animals, diets and growth performances

Forty-eight Italian Large White pigs, balanced for gender and weight, housed in 16 pens (9 m^2 concrete floor pens) with three animals in each pen, were evenly assigned to four different dietary treatments (12 pigs per diet; four replicates). The diets were similar for energy and protein levels with the same lysine/digestible energy ratio. The composition of the diets is shown in Table 1. The control group (C) received barley/soybean diet. In the three treatment (linseed) groups (experimental groups), 5% of barley was substituted for 5% of extruded linseed, either unsupplemented (L) or supplemented with a synthetic antioxidant complex (LSA) containing 200 ppm of α-tocopheryl acetate and 0.21 ppm of selenium (supported on calcium carbonate), or added with 5 g/kg of vegetal mix rich in natural antioxidants (LNA), providing 3 g/kg of grape skin extract and 2 g/kg of oregano extract, adhering to the amounts suggested by the manufacturer for food supplementation. Water was always available. The trial lasted for 104 days starting from an average live body weight (LBW) of 79.9 ± 5.8 kg (6 months of age), till slaughter at 150.5 ± 9.9 kg LBW. From starting weight and up to 113 ± 10.6 kg LBW, the subjects were fed at 7.5% of metabolic weight, calculated as $LBW^{0.75}$; thereafter, till slaughtering, the pigs were fed at 8.5% of $LBW^{0.75}$.

The natural extracts from grape skin are normally used as supplement, nutraceutical, or for food colouring; the total amount of polyphenols in the antioxidant mix of LNA group was 14.3 g/L expressed as gallic acid

Table 1. Ingredients (%), proximate composition (% on dry matter basis) and fatty acid composition (% of total fatty acids) of dietary treatments.

		C		L		LSA		LNA	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Ingredients									
Extruded linseed	%	0.00	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Barley meal	%	85.50	91.00	80.50	86.60	80.30	86.40	80.50	86.60
Soybean meal	%	11.00	5.50	11.00	5.00	11.00	5.00	11.00	5.00
L-Lysine	%	0.31	0.29	0.30	0.29	0.30	0.29	0.30	0.29
DL-Methionine	%	0.06	0.04	0.06	0.03	0.06	0.03	0.06	0.03
L-Threonine	%	0.05	0.04	0.05	0.03	0.05	0.03	0.05	0.03
Calcium carbonate	%	1.18	1.13	1.19	1.15	0.89	0.85	1.19	1.15
Dicalcium phosphate	%	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt (NaCl)	%	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin/mineral pre-mix[1]	%	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit E + Se pre-mix[2]	%	—	—	—	—	0.50	0.50	—	—
Vegetal ext. (grape skin + oregano)[3]	%	—	—	—	—	—	—	0.3+0.2	0.3+0.2
Proximate composition									
Dry Matter (DM)	%	88.30	89.50	88.60	89.80	88.70	89.90	88.80	90.00
(on DM basis)									
Digestible energy	MJ/kg	13.35	13.26	13.63	13.54	13.60	13.52	13.63	13.54
Crude protein	%	16.87	12.55	17.89	13.20	17.98	13.58	17.93	13.03
Crude fat	%	2.00	1.73	4.30	3.86	4.21	4.00	4.41	3.98
Crude fibre	%	4.76	4.50	4.91	5.08	5.01	4.65	5.26	4.97
Ashes	%	5.87	4.50	5.86	5.89	6.26	5.98	6.15	5.40
Fatty acid (FA) composition	**% (of total FAs)**								
C14:0	%	0.47	0.39	0.25	0.21	0.25	0.22	0.26	0.22
C16:0	%	29.01	24.25	18.13	15.20	17.78	15.59	18.80	15.31
C16:1	%	0.49	0.34	0.17	0.15	0.17	0.17	0.02	0.15
C18:0	%	2.03	1.51	4.00	3.18	3.88	3.34	4.16	3.23
C18:1n-9	%	14.92	13.50	20.60	18.12	20.24	18.45	21.29	18.26
C18:2n-6	%	47.55	53.67	33.50	34.69	33.91	34.09	32.52	34.47
C18:3n-3	%	4.77	5.70	22.83	28.02	23.25	27.73	22.38	27.95
C20:1	%	0.74	0.64	0.53	0.41	0.52	0.42	0.57	0.41

C: control group; L: experimental group with 5% of extruded linseed; LSA: experimental group with 5% of extruded linseed, 200 ppm vitamin E + 0.21 ppm selenium; LNA: experimental group with 5% of extruded linseed and vegetal extract 5 g/kg of feed (3.00 g of grape skin extract + 2.00 g of oregano extract).
1st = feed administered from an average weight of 79.9 to 113.4 kg (growing period); 2nd = feed administered from an average weight of 113.4 kg to the slaughter (finishing period).
[1]Vitamin/mineral: Providing the following nutrients (per kg diet as-fed): vitamin A, 15.000 IU; vitamin D3, 2.000 IU; vitamin E (α-tocopheryl acetate), 50 mg; vitamin K, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B5, 15 mg; vitamin B6, 4 mg; vitamin B12, 0.036 mg; niacin, 25 mg; folic acid, 1 mg; biotin, 0.15 mg; choline, 346 mg; Cu, 15 mg; Fe, 150 mg; Mn, 25 mg; Co, 0.4 mg; I, 1.5 mg; Zn, 100 mg; and Se, 0.1 mg.
[2]Vitamin E + selenium: Providing the following nutrients (per kg diet as fed): vitamin E (α-tocopheryl acetate), 200 mg, and Se, 0.21 mg, supported on calcium carbonate.
[3]Vegetal extract was added directly to the water of the diet and not mixed with the complete feed.

equivalent (GAE). A complete characterisation of phenolic compounds of vegetal extracts used is reported in a previous paper (Martini *et al.*, 2020). Grape skin extract was supplied by Enocianina Fornaciari s.n.c. (Reggio Emilia, Italy) and oregano extract was supplied by Phenbiox s.r.l. (Bologna, Italy). All diets were distributed in wet form (water:feed ratio of 3:1) and the vegetal extract mix was diluted in the water of the diet. During the trial, the average daily feed intake (ADFI) per pen was monitored, and the feed conversion rate (FCR) per pen was calculated.

Slaughtering and sampling

The pigs were weighed individually after an overnight fasting, and slaughtered in a commercial abattoir by exsanguination after electrical stunning, in agreement with the Council Regulation (EC) No. 1099/2009 on the protection of animals at the time of killing. Each carcass, after slaughtering, was graded in agreement with EUROP grid carcass grading, using Fat-o-Meater device (MIPAAF, 2018). The hot carcasses were weighed, the pH_1 (45 min *post-mortem*) value at the last rib level was measured and the hot carcass yield was calculated as hot carcass weight (kg)/slaughter LBW (kg)×100. Subsequently, each carcass was dissected into commercial cuts, which were weighed and cold-stored at 0–4°C for about 24 h. At 24 h *post-mortem*, the refrigerated LTL muscle from each half left carcass was transported to laboratory and sliced in four subsamples: the first to be used for pH_2 (24 h *post-mortem*), colour and drip loss analysis; the second for evaluation of oxidative stability, moisture, intramuscular lipid and protein content of raw meat; the third one for cooking loss and shear force analysis and oxidative stability of cooked meat; and the last one was vacuum-packed (Elegen, Reggio Emilia, Italy) and stored at –20°C until lipid extraction for fatty acid analysis.

Instrumental analysis

At 24 h *post-mortem*, pH values and colour parameters were measured directly on the fresh muscle. The pH value was recorded using a portable Crison pH-meter equipped with a Xerolite electrode (Crison Instruments, Alella, Spain). Meat colour was measured by Minolta CM-600d spectrophotometer (Konica Minolta Holdings Inc., Osaka, Japan) using illuminant D65 and an 8-mm diameter aperture. After calibration with a white calibration plate, five different points on each sample were analysed and the measurements were averaged. The results were expressed as the CIE Lab three coordinates: L^* – 'lightness', a^* – 'redness', and b^* – 'yellowness'. Further, Chroma (C^*), the expression of saturation index and colour intensity, was calculated as $\sqrt[2]{a^{*2}+b^{*2}}$, and the Hue angle (H^*) was calculated as arctan (b^*/a^*).

Drip loss (%) was evaluated on LTL muscles (starting at 24 h *post-mortem*) according to Honikel (1998, slightly modified). To evaluate cooking loss, a sample (approx.100 g) of LTL muscle was vacuum-packed and cooked in a water bath till the core temperature reached 70°C. After cooling, the samples were weighed, and the cooking loss was calculated as the percentage of initial sample weight. The Warner–Bratzler shear force (WBSF) was determined on cooked samples according to Honikel (1998). Briefly, from each LTL-cooked muscle, six cylindrical cores (Ø, 1.50 cm) were cut parallel to the longitudinal orientation of muscle fibres. The measurements were averaged, and the peak force was expressed as kilogram. The working conditions of the Zwick Z50 kN testing machine (model BT1-FB050TN, Zwick Roell, Kennesaw, GA, USA) were as follows: 1-kN load cell equipped with a V-shaped blade with a triangular hole of 60°; and a constant speed of 250 mm/min.

Lipid oxidation analysis

The lipid oxidation of fresh and cooked LTL samples was evaluated in duplicate according to Siu and Draper (1978, slightly modified). Minced sample, 2.5 g, was blended and homogenised with 12.5 mL of distilled water for 2 min at 9,500 rpm using an Ultra-Turrax tissue homogenizer (IKA, Germany). Before centrifugation, at 2,000 rpm at 4°C for 20 min, 12.5 mL of 10% trichloroacetic acid (TCA) solution (Sigma-Aldrich, Milan, Italy) was added. The supernatant was collected after decantation through a paper filter (Whatman No. 541), and 4 mL of clear filtrate was transferred into 15-mL pyrex tubes; 1-mL 0.06 M 2-thiobarbituric acid (TBA, Sigma-Aldrich, Milan, Italy) was added and the samples were kept for 90 min in a water bath at 80°C; the samples were cooled before reading. At the same time, the blank was run (2-mL distilled water + 2-mL TCA solution + 1-mL TBA). Absorbance at 532 nm was measured against blank sample using a Jasco spectrophotometer (Model V550, UV/VIS, Tokyo, Japan). Using 1,1,3,3 tetraethoxypropane (TEP, Sigma-Aldrich, Milan, Italy) as a standard, thiobarbituric acid reactive substances (TBARS) was expressed as milligram of malondialdehyde (MDA) per kilogram of muscle.

Chemical composition of fresh meat and feed

The analyses of moisture, ether extract and crude protein were performed on LTL muscle according to the Association of Official Analytical Chemists methods (AOAC, 1995). The results were expressed as percentage of wet matter.

Analyses for the determination of proximate composition of feeds (dry matter, ash, crude protein, crude fat and crude fibre) were carried out according to the AOAC methods (AOAC, 1995) and the results were expressed on dry matter basis. Energy values of feeds were calculated according to Sauvant *et al.* (2004).

Fatty acid profile of fresh meat

Total lipids from LTL muscle were extracted according to the Folch *et al.* (1957) method. According to

Ficarra *et al.* (2010), 25 mg of lipid extract was methylated with methanolic potassium hydroxide (KOH) solution 2N (KOH supplied by Carlo Erba, Milan, Italy, and methanol supplied by ITW Reagents, Barcelona, Spain) and an aliquot of tridecanoic acid (C13:0) (Larodan Fine Chemicals AB, Malmö, Sweden) was added as internal standard. For determining the FA profile, TRACE™ Gas Chromatograph (GC) Ultra (Thermo Electron Corporation, Rodano, Milano, Italy) equipped with Flame Ionization Detector, a PVT injector and TR-FAME column (30-m long, 0.25-mm i.d., 0.2-µm film thickness), supplied by Thermo Scientific (Rodano, Milano, Italy), was used. Methylated sample, 1 µL, was injected into GC with a split flow rate of 10 mL/min, operating at a constant flow of 1 mL/min of helium as a carrier gas. Detector and injector had the same operating temperature of 240°C. After 2 min, the program temperature was increased at a rate of 4°C per minute from 140°C to 250°C and maintained for 5 min. The Chrom-card software (version 2.3.3, Thermo Electron Corporation Rodano, Milano, Italy) was used to record, identify and integrate the peaks of fatty acid methyl esters (FAMEs). To identify the retention period of FAMEs, a solution of standard FA mixed with the known quantity of standard was used (Supelco® 37 Component FAME mix, PUFA standard n.2, Animal Source, Supelco, Bellafonte, PA, USA, and single FAMEs standard, Larodan, Fine Chemicals AB, Malmö, Sweden). The amount of each FAME was expressed as FAME relative percentage with respect to the total amount of FAMEs.

Moreover, atherogenic index, AI = [C12:0 + (4 × C14:0) + C16:0] / [n-6 PUFA + n-3 PUFA + monounsaturated FA (MUFA)] (Ulbricht and Southgate, 1991), and thrombogenic index, TI = [C14:0 + C16:0 + C18:0] / [(0.5 × MUFA) + (0.5 × n-6 PUFA) + (n-3 PUFA / n-6 PUFA)] (Ulbricht and Southgate, 1991), were calculated.

Statistical analysis

The statistical analysis was performed using the mixed model procedure of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included dietary treatments, gender and their interactions as fixed effects and pen as a random effect. The interactions were not statistically significant; therefore, they were removed from the statistical model. Live performance data as average daily gain (ADG), ADFI, FCR, and slaughter weight were covariate for starting LBW. Moreover, for ADFI and FCR, the pen was considered as an experimental unit. Carcass weight and dressing percentage were covariates for slaughter weight, while the fatty acid composition was a covariate for intramuscular fat content (IFC). When a significant ($P < 0.05$) treatment effect was observed, Tukey's multiple comparison test was performed to compare mean values.

Results and Discussion

Performance and carcass characteristics

The experimental diets did not influence ADG and slaughter LBW ($P > 0.05$) (Table 2).

These results confirm the results reported by Corino *et al.* (2008) in pigs fed with a diet containing 5% of extruded linseed and slaughtered at 100 or 160 kg, and Kouba *et al.* (2003) in pigs fed for 20, 60 or 100 days with a diet containing 6% of whole crushed linseed. Moreover, Juàrez *et al.* (2010), feeding pigs with 5%, 10% or 15% of extruded flaxseed, reported no statistical differences on ADG and final LBW but found a slight improvement of feed efficiency with an increased dietary level of flax. In our study, the control group showed the highest and the LNA group the lowest FCR values ($P < 0.05$). The

Table 2. Effect of dietary treatment and gender on live performance (data covariate for initial LBW).

	Dietary treatments					Gender		
	C	L	LSA	LNA	SEM	Gilts	Barrows	SEM
Initial LBW (kg)	77.4	82.3	80.1	79.7	2.59	79.8	79.9	1.64
ADG (kg)	0.67	0.68	0.68	0.70	0.035	0.70	0.68	0.025
ADFI (kg/day)	2.50^{a}	2.46^{ab}	2.46^{a,b}	2.42^{b}	0.019	—	—	—
FCR (kg/kg^{-1})	3.81^{a}	3.51^{b}	3.59^{b}	3.47^{b}	0.066	—	—	—
Slaughter LBW (kg)	148.9	150.9	150.6	151.7	2.52	151.5	149.6	1.86

C: control group; L: experimental group with 5% of extruded linseed; LSA: experimental group with 5% of extruded linseed, 200 ppm vitamin E + 0.21 ppm selenium; LNA: experimental group with 5% of extruded linseed and vegetal extract 5 g/kg of feed (3.00 g of grape skin extract + 2.00 g of oregano extract).
SEM: standard error of mean values; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion rate (ADFI/ADG); LBW: live body weight.
Feed conversion ratio: Pens were considered as experimental units.
^{a,b}Different letters in the same line indicate statistically different mean values ($P < 0.05$).

ADFI was higher in the control group with statistical differences ($P < 0.05$) only when compared with the LNA group (2.50 vs. 2.42 kg*day^{-1}). We could assume that the high content of polyphenols was the main reason for these results. Fiesel *et al.* (2014) stated that the presence of plant products in growing pigs' diet improves the gain:feed ratio, since polyphenols not only cause alteration in the microbial composition of the gut but also exert anti-inflammatory action.

In our study, dietary n-6:n-3 PUFA ratios were 9.8:1 in the control group and averaged as 1.35:1 in linseed groups (data not reported in the table). The groups with lower n-6:n-3 PUFA ratios showed slight improvement in feed efficiency ($P < 0.05$), and this result agrees with Duan *et al.* (2014) and Li *et al.* (2015). The authors stated that n-6:n-3 ratios of 1:1 and 1:5 compared with 10:1 improved feed efficiency of finishing pigs. These authors assumed that improvement in feed efficiency could be due to the anti-inflammatory effect of n-3, which spares energy and nutrients for tissue deposition.

Gender had no effect ($P > 0.05$) on any of the parameters evaluated. Since feed intake was recorded per pen, and both sexes were housed in each pen, the gender-wise statistical analysis of ADG and FCR was not possible.

Inclusion of dietary linseed (Table 3) did not affect ($P > 0.05$) carcass characteristics, as already observed in previous studies (Kouba *et al.*, 2003; Karolyi *et al.*, 2012).

Although differences were not statistically significant, the backfat thickness was slightly higher in the control group, and the lean meat percentage was lesser than in the linseed-fed groups (33.5 mm vs. avg. 30.9 mm, and 50.2% vs. avg. 51.4%). The same trend was recorded by Duan *et al.* (2014), who reported a significant decrease in adipose tissues and an increase in lean tissue masses with a decrease in dietary n-6:n-3 ratio from 10:1 to 1:1.

The effect of gender was significant only on the percentage of perirenal fat, higher in barrows (1.95% vs.1.59%; $P < 0.05$).

Table 3. Effect of dietary treatment and gender on carcass traits (carcass weight and dressing percentage were covariate for slaughter weight, while all carcass traits were covariate for hot carcass weight).

	Dietary treatments					Gender		
	C	L	LSA	LNA	SEM	Gilts	Barrows	SEM
Hot carcass weight (kg)	124.82	129.26	127.03	127.75	2.493	128.11	126.32	1.765
Hot carcass yield (%)	85.02	84.59	84.28	84.23	0.503	84.36	84.70	0.359
Backfat thickness (mm)	33.53	31.09	30.72	30.93	1.615	31.45	31.68	1.109
Lean meat content (%)	50.23	51.33	51.31	51.41	0.815	51.08	51.06	0.278
Lean cuts (%)[1]								
• Thigh	26.89	26.73	27.06	27.17	0.314	27.09	26.84	0.222
• Loin	18.61	17.62	18.02	17.62	0.369	18.00	17.94	0.259
• Neck	7.90	8.17	8.00	7.79	0.249	8.06	7.87	0.169
• Shoulder	14.48	14.52	14.15	14.34	0.182	14.35	14.40	0.128
• Total lean cuts	67.90	67.03	67.23	66.92	0.480	67.55	66.99	0.340
Adipose cuts (%)[1]								
• Backfat	4.50	5.06	5.22	5.53	0.285	5.17	4.98	0.200
• Belly	13.38	13.47	13.49	12.98	0.389	13.37	13.29	0.275
• Jowl	6.35	6.49	6.78	6.69	0.240	6.49	6.67	0.165
Perirenal fat	1.73	1.74	1.81	1.81	0.115	1.59[b]	1.95[a]	0.080
Total adipose cuts	25.94	26.77	27.31	26.98	0.513	26.54	26.96	0.363
Others (head, feet, tail) (%)[1]	6.13	6.20	5.46	6.10	0.580	5.91	6.05	0.410

C: control group; L: experimental group with 5% of extruded linseed; LSA: experimental group with 5% of extruded linseed, 200 ppm vitamin E + 0.21 ppm selenium; LNA: experimental group with 5% of extruded linseed and vegetal extract 5 g/kg of feed (3.00 g of grape skin extract + 2.00 g of oregano extract).
SEM: standard error of mean values.
[1]Percentage of hot carcass weight.
[a,b]Different letters in the same line indicate statistically different mean values ($P < 0.05$).

Raw and cooked meat characteristics

The effects of dietary treatment and gender on chemical and physical characteristics of LTL muscle are shown in Table 4.

In agreement with Corino *et al.* (2014) and Minelli *et al.* (2020), the pH values of LTL muscle were not influenced by dietary treatments. The colour parameters a^*, b^*, C^*, and H^* were the only parameters affected by dietary treatments. The control group showed higher a^* (3.16 vs. 1.88), b^* (12.40 vs. 11.59) and C^* (12.88 vs. 11.79) values but lower H^* (76.04 vs. 81.01) values with respect to the LSA group ($P < 0.05$). The control group yielded redder and yellower meat than the LSA group, whilst the other two diets with linseed showed intermediate and not statistically different values. Probably, vitamin E might have affected variation of colour parameters. Our data conflicted with the results of Hasty *et al.* (2002), who reported a tendentially ($P > 0.05$) linear increase of b^* value with increasing levels of dietary α-tocopheryl acetate, while Asghar *et al.* (1991) observed that a dietary supplementation of vitamin E at a level of 200 IU/kg led to an increase in the redness of pork without affecting yellowness. In general, we found that all linseed groups have tendentially lower a^*, b^* and C^* values and higher H^* value, and this indicated that extruded linseed could produce tendentially decoloured meat. The moisture, ether extract, drip loss, MDA and protein content of raw meat, and shear force and MDA content in cooked meat were not affected by dietary treatments.

Oxidative stability during meat storage and cooking is an important factor for both shelf-life and consumer safety. In this research, the MDA content increased by about four times with the cooking process, but no difference ascribable to diet or sex was found in raw or cooked meat, although unsupplemented extruded linseed showed the highest value ($P > 0.05$). These results confirmed the previous findings in pigs fed with similar diets (Minelli *et al.*, 2020).

Yet by imposing more challenging experimental conditions, other authors obtained quite different results. Among these authors are Botsoglou *et al.* (2012), who evaluated pork chops containing lipids with far higher proportions of n-6 and n-3 PUFA than our LTL samples. They observed that lipid oxidation in both raw and cooked chops was significantly alleviated in the samples obtained from the subjects that had received a dietary supplementation of α-tocopheryl acetate, 200 mg/kg feed.

Table 4. Effects of dietary treatment and gender on chemical and physical characteristics of raw and cooked *Longissimus thoracis et lumborum* muscle.

	Dietary treatments					Gender		
	C	L	LSA	LNA	SEM	Gilts	Barrows	SEM
pH_1	6.49	6.37	6.37	6.37	0.096	6.34	6.47	0.062
pH_2	5.60	5.55	5.58	5.54	0.020	5.55	5.58	0.014
L^*	52.78	52.69	53.34	53.20	0.755	53.66	52.34	0.533
a^*	3.16^{a}	2.71^{a,b}	1.88^{b}	2.07^{a,b}	0.379	2.02^{b}	2.89^{a}	0.268
b^*	12.40^{a}	11.94^{a,,b}	11.59^{b}	11.92^{a,b}	0.277	11.85	12.08	0.196
Chroma (C^*)	12.88^{a}	12.35^{a,b}	11.79^{b}	12.16^{a,b}	0.312	12.08	12.51	0.221
Hue angle (H^*)	76.04^{b}	77.80^{a,b}	81.01^{a}	80.51^{a,b}	1.672	80.73^{a}	76.95^{b}	1.18
Drip loss (%)	2.45	3.37	2.89	2.63	0.464	3.05	2.62	0.329
Cooking loss (%)	21.88	23.23	21.15	20.95	0.985	21.34	22.27	0.698
MDA, raw meat (mg/kg)	0.104	0.115	0.083	0.164	0.044	0.094	0.138	0.116
MDA, cooked meat (mg/kg)	0.401	0.501	0.401	0.380	0.0505	0.393	0.448	0.0358
Moisture (%)	68.98	68.24	67.98	68.75	0.655	68.65	68.33	0.461
Ether extract (%)	1.62	1.58	1.39	1.75	0.158	1.40^{b}	1.78^{a}	0.109
Protein (%)	23.40	23.62	23.81	22.88	0.344	23.42	23.44	0.243
WBSF (kg)	6.58	6.40	6.33	5.93	0.366	6.28	6.34	0.259

C: control group; L: experimental group with 5% of extruded linseed; LSA: experimental group with 5% of extruded linseed, 200 ppm vitamin E + 0.21 ppm selenium; LNA: experimental group with 5% of extruded linseed and vegetal extract 5 g/kg of feed (3.00 g of grape skin extract + 2.00 g of oregano extract).
SEM: standard error of mean values; MDA: Malondialdehyde; WBSF: Warner Bratzler Shear Force.
^{a,b}Different letters in the same line indicate statistically different mean values ($P < 0.05$).

Further, Martini *et al.* (2020), evaluating a subsample of this research, reported in meat grilled at 140°C for 5 min a dramatic increase of about eight times in MDA with respect to raw meat. The sharpest increase was found in the L group that showed statistically different results from all other groups, while no difference was found in C, LSA and LNA groups.

Thus, rearing and processing conditions play a pivotal role in the oxidative stability of meat enriched with n-3 PUFA. However, in our experimental conditions, the natural antioxidants were as effective as the synthetic ones in preventing formation of advanced lipid oxidation end products.

All groups showed an MDA content lower than 1.0 mg/kg of meat, which is considered the maximum acceptable threshold for rancidity (Rahman *et al.*, 2015).

Regarding the effect of gender on colour parameters, the redness value was higher in barrows than in gilts (2.89 vs. 2.02; $P < 0.05$) and H^* was lower in barrows (76.95 vs. 80.73; $P < 0.05$). Our results are in agreement with previous studies (Alonso *et al.*, 2009; Daza *et al.*, 2018). These authors reported that a^* value was directly related to myoglobin content that was higher in barrows than in gilts, and consequently their meat resulted redder globally.

LTL muscle in barrows had higher content of ether extract (1.78% vs. 1.40%; $P < 0.05$). This matched with other findings (Alonso *et al.*, 2009; Lo Fiego *et al.*, 2010; Daza *et al.*, 2018) and was largely expected, given that castration promoted the intramuscular fattening of meat (Barton-Gade, 1987).

Intramuscular fatty acid composition

Dietary treatments did not significantly affect the proportion of total SFA and MUFA ($P > 0.05$) (Table 5).

Among SFA, the percentage of 12:0 and 16:0 tended to be lower in the control group but statistically different ($P < 0.05$) from the LSA group (–0.01 and –0.95 percentage points, respectively). Among MUFA, 17:1 tended to be higher in the control group but statistically different from the L group (+0.06; $P < 0.05$). Irrespective of the type of antioxidant used, inclusion of extruded linseed in the diet increased ($P < 0.05$) the proportion of all n-3 PUFA except DHA, in agreement with Guillevic *et al.* (2009) and Minelli *et al.* (2020). Further, DHA was lower ($P < 0.05$) in the LSA group than in the L group (0.07% vs. 0.10%, respectively). Moreover, linseed dietary inclusion reduced ($P < 0.05$) the n-6:n-3 PUFA ratio and the proportion of all n-6 PUFA, except 18:2 n-6 and 20:2 n-6 ($P > 0.05$). The total content of PUFA was lowest in the LSA group and statistically different ($P < 0.05$) compared with the L group. The thrombogenic index (TI) decreased ($P < 0.05$) with linseed feeding but only in the L group compared with the control group.

In general, main variations in FA composition ascribable to linseed feeding are as follows: an increase of n-3 PUFA, mainly ALA and its derivates EPA and DPA, originated from elongase and desaturase reactions; decrease of n-6 PUFA, mainly 22:4 n-6, probably accounted for by the higher affinity of Δ6 desaturase for n-3 substrates (Lee *et al.*, 2016) and decrease of n-6:n-3 ratio below 4, the maximum threshold indicated by Simopoulos (2002) to avoid adverse health consequences. Our results agree with previous works (Riley *et al.*, 2000; Minelli *et al.*, 2020).

The FA composition of intramuscular fat did not differ significantly between LSA and LNA groups. Regarding the n-6:n-3 ratio, it did not vary in linseed-fed groups, although antioxidant supplementation led to a numerical increase of this ratio, with the highest value found in the LSA group.

This could be due to different effects of diets enriched with n-3 PUFA and natural or synthetic antioxidants in modulating the expression of pig skeletal muscle genes involved in *de novo* synthesis of FA and metabolism of lipids. Vitali *et al.* (2019) reported a more evident stimulation of the expression of genes involved in controlling muscle metabolism when n-3 dietary PUFA are administered in association with polyphenols-enriched diet.

Pigs' gender affected the total MUFA content, higher in barrows than in gilts (47.11 vs. 45.29; $P < 0.05$), specifically 18:1 n-7 and 18:1 n-9. Moreover, barrows had the lowest amount of PUFA (14.34 vs. 16.52, $P < 0.05$) and total n-6 content (11.80 vs. 13.57; $P < 0.05$), confirming previous findings (Alonso *et al.*, 2009; Lo Fiego *et al.*, 2010; Okrouhlá *et al.*, 2013). No difference was observed for total n-3, n-6:n-3 PUFA ratio, and atherogenic and thrombogenic indices.

Conclusion

Our study confirms that 5% of dietary-extruded linseed in pig diet is a suitable means to increase n-3 PUFA content and reduce n-6:n-3 PUFA ratio in LTL muscle, an important aim from the point of view of human nutrition, without affecting live performances and carcass traits. The technological characteristics of carcass and meat were not impaired by the ameliorated PUFA ratio. The main qualitative characteristics and chemical composition of the muscle were neither affected by linseed feeding nor by inclusion of synthetic or natural

Table 5. Effect of dietary treatments and gender on fatty acid profile (% of total fatty acid) of *Longissimus thoracis et lumborum* muscle.

	Dietary treatments					Gender		
	C	L	LSA	LNA	SEM	Gilts	Barrows	SEM
C10:0 (capric)	0.12	0.11	0.12	0.11	0.005	0.11^{b}	0.12^{a}	0.004
C12:0 (lauric)	0.07^{b}	0.08^{a,b}	0.08^{a}	0.08^{a,b}	0.003	0.07^{b}	0.08^{a}	0.002
C14:0 (myristic)	1.19	1.23	1.27	1.28	0.034	1.20^{b}	1.29^{a}	0.025
C16:0 (palmitic)	23.24^{b}	23.41^{a,b}	24.19^{a}	24.00^{a,b}	0.319	23.49	23.93	0.233
C17:0 (heptadecanoic)	0.23	0.21	0.25	0.23	0.018	0.23	0.23	0.012
C18:0 (stearic)	12.66	12.69	12.94	13.11	0.354	12.95	12.75	0.258
C20:0 (eicosanoic)	0.14	0.15	0.14	0.14	0.006	0.14	0.15	0.004
C16:1 (palmitoleic)	3.18	3.07	3.10	2.96	0.124	2.94	3.22	0.090
C17:1 (heptadecenoic)	0.30^{a}	0.24^{b}	0.26^{a,b}	0.28^{a,b}	0.014	0.27	0.26	0.010
C18:1n-7 (vaccenic)	4.15	4.05	4.05	3.91	0.101	3.92^{b}	4.17^{a}	0.074
C18:1n-9 (oleic)	38.88	37.27	39.03	37.48	0.651	37.54^{b}	38.78^{a}	0.475
C20:1 (eicosenoic)	0.62	0.64	0.67	0.66	0.033	0.62	0.68	0.023
C18:2n-6 (linoleic)	9.45	9.70	8.38	9.37	0.514	9.80^{a}	8.65^{b}	0.374
C18:3n-3 (α-linolenic)	0.49^{b}	1.85^{a}	1.62^{a}	1.84^{a}	0.086	1.53	1.37	0.062
C18:3n-6 (γ-linolenic)	0.22^{a}	0.19^{a,b}	0.16^{b}	0.19^{a,b}	0.014	0.20	0.18	0.010
C20:2n-6 (eicosadienoic)	0.23	0.24	0.21	0.24	0.010	0.24^{a}	0.22^{b}	0.007
C20:3n-3 (eicosatrienoic)	0.08^{b}	0.22^{a}	0.20^{a}	0.22^{a}	0.010	0.19	0.17	0.007
C20:4n-6 (arachidonic)	3.54^{a}	2.86^{a,b}	2.03^{c}	2.37^{b,c}	0.252	2.96	2.44	0.184
C20:5n-3 (eicosapentaenoic)	0.14^{c}	0.58^{a}	0.40^{b}	0.48^{a,b}	0.046	0.44	0.36	0.033
C22:4n-6 (docosatetraenoic)	0.53^{a}	0.31^{b}	0.24^{b}	0.28^{b}	0.029	0.37	0.31	0.021
C22:5n-3 (docosapentaenoic)	0.45^{b}	0.80^{a}	0.59^{b,c}	0.69^{a,c}	0.067	0.69	0.57	0.049
C22:6n-3 (docosahexaenoic)	0.09^{ab}	0.10^{a}	0.07^{b}	0.08^{a,b}	0.009	0.10^{a}	0.07^{b}	0.006
Total saturated	37.65	37.88	38.99	38.95	0.648	38.19	38.55	0.472
Total monounsaturated	47.13	45.27	47.11	45.29	0.778	45.29^{b}	47.11^{a}	0.567
Total polyunsaturated	15.22^{ab}	16.85^{a}	13.90^{b}	15.76^{a,b}	0.965	16.52^{a}	14.34^{b}	0.703
Total n-6	13.97^{a}	13.30^{c}	11.02^{b}	12.45^{a,b,c}	0.789	13.57^{a}	11.80^{b}	0.575
Total n-3	1.25^{c}	3.55^{a}	2.88^{b}	3.31^{a,b}	0.198	2.95	2.54	0.145
n-6:n-3 ratio	11.39^{a}	3.73^{b}	3.95^{b}	3.79^{b}	0.142	5.74	5.70	0.103
Atherogenic index AI	0.45	0.46	0.48	0.48	0.012	0.46	0.48	0.009
Thrombogenic index TI	1.05^{a}	0.91^{b}	1.00^{a,b}	0.95^{a,b}	0.035	0.96	0.10	0.026

C: control group; L: experimental group with 5% of extruded linseed; LSA: experimental group with 5% of extruded linseed, 200 ppm vitamin E + 0.21 ppm selenium; LNA: experimental group with 5% of extruded linseed and vegetal extract 5 g/kg of feed (3.00 g of grape skin extract + 2.00 g of oregano extract).
SEM: standard error of mean values.
Atherogenic index, AI = [C12:0 + (4 × C14:0) + C16:0] / [n-6 PUFA + n-3 PUFA + MUFA] (Ulbricht and Southgate, 1991).
Thrombogenic index, TI = [C14:0 + C16:0 + C18:0] / [(0.5 × MUFA) + (0.5 × n-6 PUFA) + (n-3 PUFA / n-6 PUFA)] (Ulbricht and Southgate, 1991).
^{a,b}Different letters in the same line indicate statistically different mean values ($P < 0.05$).

antioxidants. Absence of further improvement with the addition of natural antioxidants may be due to the low quantity used in this study, based on the quantities used in human diets.

Further research should investigate the effects of higher levels of natural antioxidants, added to n-3 PUFA-enriched diets, on pork quality under different storage conditions and cooking methods.

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