

Effect of an extruded linseed diet on meat quality traits in Nero Lucano pigs

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Abstract

The study aimed to evaluate the effect of a diet containing extruded linseed (*Linum usitatissimum* L.) on growth performances, carcass traits and meat quality in Nero Lucano pigs. Sixteen male piglets were weaned at about 50 days of age and divided into two homogeneous groups fed a control diet (C) or a diet containing 3% extruded linseed (EL). The trial lasted 32 weeks and pigs were slaughtered at 9 months of age. Samples from the *Longissimus dorsi* (Ld) muscle were analysed to assess physical and chemical parameters and intramuscular fatty acid composition. Average daily gain, feed intake and feed conversion ratio were not affected by the diet. Pigs fed the C diet showed a significantly greater slaughter weight in comparison to the EL group (116.50 vs 108.25 kg). Meat pH and chemical composition were not influenced by the diet. No differences were found for shear force (WBS) in raw meat, while cooked meat of the EL group showed a lower WBS value. The EL diet significantly lowered total SFA and increased total PUFA, of both n-6 and n-3 series. Among the n-6, significant differences between dietary treatments were found for linoleic acid. As for the n-3 series, EL feeding significantly enhanced linolenic acid, EPA, DPA and DHA. Feeding EL also significantly lowered the PUFA/SFA and n-6/n-3 ratios and the AI and TI, with benefits for human health. In conclusion, dietary supplementation with 3% EL didn't affect growth performance while it improved the fatty acid profile of meat.

Keywords: fatty acid profile, feeding, flaxseed, pork

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Introduction

There is a growing demand by the modern consumer for meat containing less fat and lower levels of saturated fatty acids, which are considered to increase risk for coronary heart disease (CHD) (Department of Health, 1994). Research in the last twenty years has focused on improving the balance between polyunsaturated fatty acids (PUFA) and saturated fatty acids (SFA) in animal products, since there has been increased recognition of health benefits of PUFA in general, and of n-3 fatty acids in particular, which are essential for humans (Simopolous, 2001). Years of recommendations to reduce red meat consumption have not lowered the incidence of diseases related to dietary fat. Recently, the dogma that meat consumption should be limited in human diets because of its fatty acid composition has become controversial (Vahmani *et al.*, 2015).

In monogastric animals it is possible to enhance the concentration of beneficial n-3 fatty acids in the muscle since meat lipids reflect the nature of dietary fat (Kouba & Mourot, 2011), on the contrary to ruminants in which a well-documented hydrogenating action of rumen microorganisms on dietary fatty acids occurs (Raes *et al.*, 2004). Several lipid sources have been tested for pig feeding with the aim to improve the n-6/n-3 ratio of meat that should be 4-5 or less according to the latest nutritionist recommendations (Duru, 2019). Matthews *et al.* (2000) reported that feeding whole linseed to pigs increases the n-3 fatty acids in various tissues without adverse effects on growth or meat quality. Supplementation with 3% dietary linseed oil enhanced the daily gain of growing pigs and increased the C18:3n-3 concentration in the adipose tissue (Nguyen *et al.*, 2004). However, PUFA enriched meats are more susceptible to oxidation which may be

limited by antioxidants. D'Arrigo *et al.* (2002) found that diet enrichment with linseed oil (3%) and α -tocopherol acetate produced a healthier fat and prevented lipid oxidative deterioration.

Many aspects of meat production quality are related to genotype, sex, and age of the animals and the breeding system that produces them. In Southern Italy, farmers in marginal areas practice environmentally friendly production systems using native genotypes that are well adapted to the environment and able to exploit the feed resources available on the territory (Maiorano *et al.*, 2007; Tarricone *et al.*, 2010; Marsico *et al.*, 2011; Pugliese & Sirtori, 2012). The Nero Lucano pig is a swine breed that is native to Southern Italy and bred in the interior of the Lucanian region. These pigs are of moderate size, black-cloaked, adaptable to different environments and able to use the poor feed resources present in wild landscapes. This breed has been slowly undergoing genetic erosion over time due to the increasing interest of breeders towards foreign germplasm that is faster growing, more efficient and has better carcass conformation and meat yield. However, meat of this native breed is very much appreciated by the consumer and also for producing salami, since it is considered to be wholesome and flavoursome. However, there are very few studies that characterize the performance and meat quality traits of the Nero Lucano pig (Marsico *et al.*, 2011). Thus, the aim of our study was to investigate the effects of a diet containing extruded linseed (*Linum usitatissimum* L.) on *in vivo* growth, carcass traits, meat quality and muscle fatty acid composition in this autochthonous swine breed.

Materials and Methods

The study was carried out at the farm "Masseria Pian del Camino" located in Albano di Lucania (Latitude 40°36'03.7"N, Longitude 16°03'00.1"E, 899 m asl) from October to June 2018. Animals were cared for following the European Union guidelines (Directive 2010/63/EU).

Sixteen male Nero Lucano piglets from two litters were weaned at about 40 days and then divided into two groups of eight animals each homogeneous for age (47 ± 3 days old) and initial live weight (12.6 ± 0.2 kg). The pigs were housed indoors in a shed; each group was kept in a single pen having a floor covered by straw and a surface of 24 square meters. Each box had front access to an outdoor paddock of 50 square meters. The pigs were assigned to one of the following dietary treatments: a commercial pelleted feed (Control diet, C) and a diet containing 3% extruded linseed on an as fed basis (EL). The diets were formulated in order to be iso-energetic, iso-proteic, and iso-lipidic (INRA, 1989). Feed and chemical composition and the nutritive value of the feedstuffs are shown in Table 1.

Pigs had free access to water and feed was offered daily each morning at a rate of 110% of *ad libitum* intake calculated by weekly weighing-back of refusals. Every day, after the pigs had finished eating, the remaining pellet feeds were weighed to determine the daily feed intake of each group. The individual body weights were recorded at the beginning of the trial (day 0) and every week until slaughtering day (day 224). Feed samples were taken monthly and stored at -20 °C until analysis. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR, kg food ingested daily/kg daily body growth) were calculated from these data.

Samples of each diet were ground in a hammer mill with a 1 mm screen and analysed in triplicate using AOAC (2004) procedures: dry matter (DM) (Method 934.01), ether extract (EE) (Method 920.39), ash (Method 942.05), crude protein (CP) (Method 954.01), and crude fibre (CF) (Method 945.18). The fatty acid profile of each diet was determined using the method described below for meat.

Thirty-two weeks after the beginning of the trial when the pigs were approximately 9 months old they were slaughtered. After an on-farm fasting period of 8 h, the pigs were transported to the abattoir. They were laired for 4 h with free access to water. Pigs were electrically stunned and following exsanguination, the carcasses were scalded, dehaired and eviscerated. Pigs were weighed at slaughter and hot carcass weight was recorded within 45 minutes after slaughter, in accordance with the Italian ASPA methods (1991).

Within 45 minutes after slaughter the pH of the *Longissimus dorsi* (*L. dorsi*) muscle ($\text{pH}_{45 \text{ min}}$) was measured using a portable instrument (Hanna Instruments HI 9025, Woonsocket, RI) with a penetrating glass electrode (FC 230C, Hanna Instruments) and performing two-point calibration (pH 7.01 and 4.01). After 24 hours of refrigeration at $0 - 4$ °C the carcasses were weighed again to calculate the cold yield and the pH of the *L. dorsi* muscle was again recorded ($\text{pH}_{24 \text{ h}}$).

The carcasses were divided in half at the midline and the right side was dissected into the following cuts: head, jowl, neck with bone, shoulder, loin, brisket, belly, ham, kidney, flare fat, feet and tail. The ham, loin and shoulder were dissected into their tissue components (lean, separable fat, bone, and rind).

Table 1 Feed ingredients (g/kg as fed basis), chemical (% dry matter basis) and FA composition (% FA methyl esters) of the diets

Ingredients (g/kg as-fed basis)	Control feed	Extruded linseed feed
Corn	376.5	375.0
Barley	230.0	230.0
Soybean meal (43.4% CP ¹)	169.0	154.0
Wheat flour shorts	115.0	115.0
Wheat middlings	40.0	40.0
Molasses	30.0	30.0
Extruded linseed	0	3.0
Limestone	14.0	14.0
Monocalcium phosphate	8.0	8.0
Sodium chloride	4.0	4.0
Lysine	1.7	1.7
Choline	0.008	0.008
Premix ²	1.0	1.0
<i>Chemical composition (DM³ basis)</i>		
DE, Mcal/kg	3.15	3.15
Moisture	10.40	10.40
Crude protein	16.28	15.99
Ether extract	3.34	3.04
Ash	6.14	6.29
Crude fibre	4.79	5.01
<i>Fatty acid composition (% fatty acid methyl esters)</i>		
C16:0	14.3	14.0
C18:0	3.1	3.0
C18:1c9 (n-9)	24.2	20.8
C18:2c9,t11 (n-6)	30.4	38.4
C18:3c6,9,12 (n-3)	3.3	18.1

¹CP: crude protein; ²1 kg of Premix contains: vitamin A 6000 IU, vitamin D3 800 IU, vitamin E 20 mg, niacin 6 mg, Ca D-pantothenate 4 mg, riboflavin 1.5 mg, vitamin K 1 mg, vitamin B6 1 mg, vitamin B1 0.8 mg, folic acid 0.25 mg, biotin 0.05 mg, vitamin B12 0.01 mg, choline Cl 600 mg, MnO 25.8 mg, ZnO 24.8 mg, FeSO₄·H₂O 76 mg, FeCO₃·H₂O 20.7 mg, CuSO₄·5H₂O 19.65 mg, Ca(IO₃)₂ 0.62 mg, Na₂SeO₃ 0.18 mg, L-lysine monohydrochloride 1.326 mg; ³DM: dry matter.

The *L. dors*i samples from the right carcass were used to measure meat colour and tenderness. The colorimetric parameters (L* = lightness, a* = redness, b* = yellowness) were determined using a Hunter Lab Miniscan™ XE Spectrophotometer (Model 4500/L, 45/0 LAV, 3.20 cm diameter aperture, 10° standard observer, focusing at 25 mm, illuminant D65/10; Hunter Associates Laboratory Inc., Reston, Virginia, USA). Three readings for each sample (measuring approximately 10x15 cm) were taken by placing the colorimeter in three different points of the lumbar surface (proximal, central and distal, at about 5 cm of distance one from another). The instrument was normalized to a standard white tile provided with the instrument before performing analysis (Y = 92.8, x = 0.3162, and y = 0.3322) (ASPA, 1996). The reflectance measurements were performed after the samples had oxygenated in air for at least 30 min, at which time the measurements were stable (Šicklep & Čandek-Potokar, 2007).

Meat tenderness was assessed on raw and cooked *L. dors*i samples by the Warner Bratzler shear (WBS) force system using an Instron 5544 Universal Testing Machine (Instron Corp., Canton, MA, USA). The samples were cylinders 25.4 mm in diameter, were assessed in triplicate and sheared perpendicularly to the muscle fibre direction (load cell 50 kg, shearing speed 200 mm/min). Peak force was expressed as kg/cm² (ASPA, 1996).

To determine the cooking loss percentage, homogeneous samples (about 5 cm thick) were cut from the *L. dors*i muscle and weighed before and after cooking performed in a ventilated electric oven at 165 °C, until an internal temperature of 75 °C was reached in the core of the sample (ASPA, 1996), as recorded by a thermocouple (Hanna Instruments, Villafranca, Padovana, Italy).

AOAC (1995) procedures were used to measure moisture, crude fat, protein and ash contents of *L. dors*i raw samples.

Total lipids were extracted from the homogenized samples (100 g) using the chloroform/methanol method described by Folch *et al.* (1957). Fatty acids were methylated using KOH and methanol (solution 2N) according to Christie (1982) and the fatty acid profile was assessed using a gas chromatograph (Shimadzu GC-17A) with a silicate glass capillary column (70% Cyanopropyl Polysilphenylenesiloxane BPX 70 of SGE Analytical Science, length = 50 m, internal diameter = 0.22 mm, film thickness = 0.25 µm). The temperature programme was as follows: 135 °C for 7 min, followed by increases of 4 °C per minute up to 210 °C and held for 2 minutes. The injector and detector temperatures were set up at 245 °C and 280 °C, respectively. Helium gas was used as carrier with a flow of 1.2 ml/min. Fatty acid were identified using a mixture of standard fatty acids (Restek Corporation, Bellefonte, PA, USA) and were expressed as percentage (wt/wt) of total methylated fatty acids.

The food risk factors of meat were determined by calculating the atherogenic (AI) and thrombogenic (TI) indices (Ulbricht & Southgate, 1991) as follows:

$$AI = [(C12:0 + 4 \times C14:0 + C16:0)] \div [\Sigma MUFA + \Sigma n-6 + \Sigma n-3]$$

$$TI = [(C14:0 + C16:0 + C18:0)] \div [(0.5 \times \Sigma MUFA + 0.5 \times \Sigma n-6 + 3 \times \Sigma n-3) / \Sigma n-6]$$

where MUFA are monounsaturated fatty acid concentrations.

The data were analysed for variance using the GLM procedure of SAS (SAS Institute Inc., Raleigh, North Carolina, USA). Data were processed by one-way ANOVA with dietary treatment as the main effect. All tables contain Least Squares Means (LSM) and the standard error of the mean (SEM). LSM were compared by the Student's t-test.

Results and Discussion

Diet had no effect on the average daily gain (490 vs 470 g, C and EL group, respectively, $P=0.63$), feed intake (2050 vs 1970 g, C and EL group, respectively, $P=0.70$) and feed conversion ratio (4.18 vs 4.19, C and EL group, respectively, $P=0.12$) in agreement with previous studies which have evaluated the effects of dietary linseed treatments on pig growth and meat quality (Okrouhlá *et al.*, 2013; De Tonnac *et al.*, 2017).

Carcass data are shown in Table 2. Pigs fed the control diet had a greater ($P < 0.05$) slaughter weight in comparison to the extruded linseed group, despite no differences having been observed for feed intake and growth rate. This may be attributable to differences in the amount of gastrointestinal contents. However, this quantity was not assessed. Zhan *et al.* (2009) reported that stimulatory effects of linseed on growth may not be apparent when the level added is less than 5%. The hot and cold dressing percentages were higher ($P < 0.01$) in pigs fed extruded linseed. Corino *et al.* (2008) did not find any differences in dressing percentage and carcass traits for pigs fed with a diet containing 5% extruded linseed and slaughtered at about 110 kg live weight. De Tonnac *et al.* (2017) also did not find any differences with regards to growth and carcass traits of pigs fed 3.6% extruded linseed. However, in lambs (Colonna *et al.*, 2011; Facciolongo *et al.*, 2018) and kids (Rotondi *et al.*, 2018) fed diets containing 3% extruded linseed significantly greater hot dressing percentages were recorded.

Table 2 Weights and dressing percentages of Nero Lucano pigs fed a control diet or a similar diet that had been supplemented with 3% extruded linseed

	Diets		SE	P-value
	Control	Extruded linseed		
Slaughter weight (kg)	116.50	108.25	10.015	0.042
Hot carcass weight (kg)	96.12	95.05	1.580	0.600
Hot dressing percentage (%)	82.51	87.80	0.558	0.0001
Cold dressing percentage (%)	78.84	83.04	0.646	0.0001

Table 3 reports the section data from the right half of the carcasses. The extruded linseed dietary treatment produced an increased proportion of feet ($P < 0.05$) and tail ($P < 0.01$), while it reduced the proportion of loin ($P < 0.05$), flare fat ($P < 0.01$), and shoulder ($P < 0.01$). Previously, in pigs fed 5% linseed and slaughtered at 110 kg, the loin weight/carcass weight ratio was increased Corino *et al.* (2008), a finding which differs from our results. These differences may be due to the lower percentage of extruded linseed used in the present study or to the genetic difference in the pigs. The carcass data recorded for the control group in this trial confirm the results of Marsico *et al.* (2011) carried out in Nero Lucano pigs.

Table 3 Carcass weight and percentages of individual cuts from the right half carcass of Nero Lucano pigs

Trait	Diets		SE	P-value
	Control	Extruded linseed		
Half carcass weight (kg)	46.60	45.25	4.391	0.126
Head	13.63	12.99	1.164	0.436
Jowl	2.27	2.31	0.579	0.938
Neck with bone	8.34	8.17	0.686	0.664
Shoulder	16.95	19.57	0.668	0.002
Loin	13.62	11.74	0.869	0.023
Brisket	1.65	1.57	0.229	0.654
Belly	10.70	11.26	1.172	0.564
Ham	28.15	28.17	1.382	0.892
Kidney	0.39	0.39	0.057	0.904
Flare fat	2.01	0.86	0.278	0.002
Feet	1.99	2.30	0.179	0.034
Tail	0.30	0.67	0.138	0.012

Several studies (Maiorano *et al.*, 2007; Juárez *et al.*, 2011; Okrouhlá *et al.*, 2013) summarized the carcass cut-out data as percentages of lean, fatty and other cuts instead of the single meat cuts. In our study, the total lean (sum of ham, loin, neck and shoulder) was 67.06 and 67.65%, respectively, for the C and EL groups, which was greater than comparable estimates of Maiorano *et al.* (2007) in Casertana pigs and of Corino *et al.* (2008) in Landrace crossbred pigs slaughtered at a similar age and weight. These results confirm that Nero Lucano is a small size, lean, and rustic pig genotype as reported also by Marsico *et al.* (2011).

Data from dissection of the ham, loin and shoulder into their tissue components (lean, bone, fat and rind) are shown in Table 4. Although no differences were found between treatments for the weight of the single meat cuts, the control diet resulted in an increased ($P < 0.01$) percentage of lean in the ham and shoulder. Feeding extruded linseed increased the percentages of fat, bone and rind in the ham ($P < 0.05$) and fat and rind in the shoulder ($P < 0.01$). Several authors reported that dietary linseed increased the lean yield of commercially important meat cuts in pigs (Corino *et al.*, 2008; Juárez *et al.*, 2011) and young bulls (Tarricone *et al.*, 2019). However, other investigators (Bee *et al.*, 2008; Bečková & Václavková, 2010), did not observe any increase in the total lean content; findings which are consistent with our results.

Table 4 Cut weights and percentages of lean, fat, bone and rind of the ham, loin and shoulder of Nero Lucano pigs

	Diets		SE	P-value
	Control	Extruded linseed		
Ham (kg)	13.09	13.47	1.864	0.786
Lean	70.85	65.88	0.976	0.001
Bone	7.30	9.44	0.914	0.016
Fat	11.19	10.55	1.015	0.405
Rind	10.66	14.14	1.805	0.035
Loin (kg)	6.37	5.62	0.978	0.318
Lean	61.12	58.02	2.130	0.085
Bone	6.2	11.21	3.845	0.115
Fat	19.01	15.41	3.218	0.165
Rind	13.68	15.37	2.080	0.294
Shoulder (kg)	8.29	9.29	0.844	0.146
Lean	71.12	59.72	2.374	0.001
Bone	10.07	9.57	0.342	0.087
Fat	7.24	12.61	1.237	0.001
Rind	11.98	18.11	3.135	0.013

Table 5 shows the physical and chemical features of meat from the *L. dorsi* muscle. Meat pH was not influenced by the diet; and the pH values of meat recorded at slaughtering and after 24 hours of refrigeration were consistent with those reported in other studies (Maiorano *et al.*, 2007; Corino *et al.*, 2008).

Table 5 Mean (\pm SE) physical and chemical data of meat from *Longissimus dorsi* muscle of Nero Lucano pigs

Trait	Diets		SE	P-value
	Control	Extruded linseed		
pH _{45 min}	6.50	6.27	0.161	0.089
pH _{24 h}	5.53	5.62	0.248	0.617
Lightness (L*)	51.31	55.34	2.133	0.037
Redness (a*)	3.03	6.31	0.528	<0.001
Yellowness (b*)	10.76	13.14	0.331	<0.001
WBS ¹ raw meat (kg/cm ²)	3.30	2.74	0.787	0.349
WBS ¹ cooked meat (kg/cm ²)	3.38	2.35	0.259	0.001
Cooking loss (%)	26.60	32.30	2.602	0.021
Moisture (%)	74.35	74.25	0.844	0.946
Protein (%)	22.31	21.95	0.401	0.261
Fat (%)	1.80	2.30	0.603	0.290
Ash (%)	1.54	1.50	0.147	0.055

¹WBS: Warner Bratzler Shear Force

Dietary linseed feeding affected meat colour features since the higher values for lightness ($P < 0.05$), redness ($P < 0.01$) and yellowness ($P < 0.01$) indicated a shift to darker red meat. Bee *et al.* (2008) and Corino *et al.* (2008) did not report effects on meat colour, while Juárez *et al.* (2011) found lower values for L, chroma and hue in pigs fed 5 - 10% flaxseed level. The effect of extruded linseed or linseed oil on meat colour features is quite controversial. Barahona *et al.* (2016) found that the inclusion of dietary whole linseed decreased hue and yellowness whereas it increased redness of beef meat, while Haak *et al.* (2008) reported no effect of duration or of time of linseed supplementation on pork meat lipid or colour oxidation. Moreover, Kasprzyk *et al.* (2015) found that the commercial Polish Landrace pig breed had a higher L* value than the autochthonous Pulawska breed which had greater redness. Franci *et al.* (2005) also reported that meat from Italian local breeds was redder than that of Large White pigs. Wood *et al.* (2003), furthermore, reported that diets aiming to enrich pig meat in PUFAs may affect colour changes related to the oxidation of red oxymyoglobin to brown metmyoglobin products, leading also to off-odours and off-flavours.

No differences were found between groups as for shear force in raw meat, while cooked meat of the extruded linseed group was more tender ($P < 0.01$) despite a higher cooking loss ($P < 0.05$). The eating quality of pork is a combination of many factors among which tenderness and juiciness are very important. Temperature and duration of cooking have a large effect on the physical properties of cooked meat. Many different experimental methods have been used to cook pork making difficult to directly compare the results. In many studies the final internal temperature is not clearly defined. The mechanical properties of meat have been thoroughly reviewed in many animal species (Bouton *et al.*, 1978; Thompson, 2002; Hoffman *et al.*, 2003; Bekhit *et al.*, 2014; Choe *et al.*, 2016) and are known to be affected by breed, age, sex, rearing system, slaughter weight, muscle, connective tissue protein, collagen content and solubility, fat content and quality, and drip loss. Although meat samples of the EL group showed only a slightly greater fat content, it may be hypothesized that the higher concentration of PUFA fatty acids in their meat and also the quality of intramuscular fat as opposed to its total content, may have affected meat texture. How the extruded linseed diet influences the muscle texture in Nero Lucano pigs still needs to be ascertained since there are no previous references for this native breed.

In this study, the chemical composition of meat was not affected by the diet. Okrouhlá *et al.* (2013) found similar results.

The fatty acid profile of meat from the *L. dorsi* is shown in Table 6. The present study confirms the results published by other authors who found that dietary supplementation with linseed is reflected in the fatty acid profile of pig muscle (Enser *et al.*, 2000; Kouba *et al.*, 2003; Guillevic *et al.*, 2009). In this study, the total amount of SFA was reduced by dietary supplementation with extruded linseed ($P < 0.05$) in agreement with Juárez *et al.* (2010). Palmitic (C16:0) and stearic (C18:0) acids were both found to be lower in the extruded linseed group when compared to the control pigs, with significant differences ($P < 0.01$) for stearic acid.

Diet did not affect the amount of monounsaturated fatty acids (MUFA) expressed as a proportion of all fatty acid methyl esters. However, feeding extruded linseed did lower the palmitoleic concentration ($P < 0.05$).

Feeding extruded linseed increased ($P < 0.01$) the concentration of total polyunsaturated fatty acids, of both the n-6 series and n-3 series. Among the n-6, differences were found for linoleic acid ($P < 0.01$) as well as for eicosadienoic (C20:2), eicosatrienoic (C20:3), and arachidonic (C20:4) acids ($P < 0.05$). These results are in agreement with Juárez *et al.* (2010) who found that feeding flax diets for increasing durations prior to slaughter led to linear increases in linoleic acid. We have not performed sensory analysis on meat. However, it may be hypothesized, following Cameron *et al.* (2000) who reported that pork flavour, flavour liking, and overall acceptability are positively correlated with the intramuscular concentration of linoleic, arachidonic and C22:4 n-6, that the increase of these fatty acids observed in our study may contribute to improve pork meat palatability and healthiness.

For PUFA of the n-3 series, dietary extruded linseed increased the proportion of linolenic acid ($P < 0.05$) along with C20:3, C20:5, C22:5, and C22:6 ($P < 0.01$). This increase in the proportion of n-3 fatty acids reflects the higher concentration of linolenic acid present in the extruded linseed diet that was fed. Our results are similar to those reported by Juárez *et al.* (2011) even though we fed a diet containing less extruded linseed. Huang *et al.* (2008) and He-Feng *et al.* (2009) also found that a greater duration of feeding linseed effectively increased the muscle PUFA content. Therefore, in our study it may be hypothesized that although we used a lower extruded linseed dietary supplementation, the long duration of the dietary treatment may have provided positive results in terms of PUFA intramuscular concentration.

Table 6 Fatty acid composition (% of total fatty acid methyl esters) of meat from the *Longissimus dorsi* muscle of Nero Lucano pigs

	Diets		SE	p-value
	Control	Extruded linseed		
Total fatty acids (g/100 g muscle)	1.62	2.07		
C14:0 (myristic)	1.71	0.90	0.282	0.074
C16:0 (palmitic)	24.68	19.39	1.596	0.058
C18:0 (stearic)	12.94	10.07	0.308	<0.001
C20:0 (arachidic)	0.31	0.25	0.043	0.332
Total SFA ²	39.99	30.73	1.761	0.010
C16:1 c9 (n-7) (palmitoleic)	2.81	2.16	0.182	0.047
C18:1 c9 (n-9) (oleic)	40.22	36.42	1.491	0.121
Total MUFA ³	46.82	44.02	1.420	0.213
C18:2 c9,c12 (n-6) (linoleic)	9.14	14.47	0.854	0.005
C20:2 c11,c14 (n-6) (eicosadienoic)	0.42	0.70	0.071	0.029
C20:4 c5,8,11,14 (n-6) (arachidonic)	0.10	0.21	0.032	0.049
Total n-6 ⁴	10.01	16.16	0.859	0.002
C18:3 c9,12,15 (n-3) (gamma-linolenic)	0.51	0.97	0.065	0.003
C20:3 c11,14,17 (n-3) (eicosatrienoic)	0.46	2.69	0.148	<0.001
C20:5 c8,11,14,17 (n-3) (EPA)	0.02	0.19	0.021	0.001
C22:5 c 4,7,10,13,16 (n-3) (DPA)	0.06	0.51	0.054	0.001
C22:6 n-3 c7,10,13,16,19 (n-3) (DHA)	0.01	0.20	0.025	0.002
Total n-3 ⁵	1.07	4.56	0.141	<0.001
Total PUFA ⁶	11.94	21.57	0.976	0.004
PUFA/SFA	0.29	0.70	0.033	<0.001
n-6/n-3	9.35	3.55	0.108	<0.001
Atherogenic index	0.62	0.44	0.035	0.013
Thrombogenic index	1.53	1.17	0.035	<0.001

²SFA: saturated fatty acids (sum of C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0)

³MUFA: monounsaturated fatty acids (sum of C16:1cis-9 + C17:1cis-10 + C18:1trans-11 + C18:1trans-9 + C18:1trans-10 + C18:1cis-9)

⁴n-6: omega 6 (sum of C18:2 c9,t11 + C18:2 c9,c12 + C18:2 t10,c12 + C18:3 c6,9,12 + C20:2 c11,14 + C20:3 c8,11,14 + C20:4 c5,8,11,14 + C22:5 c4,7,10,13,16)

⁵n-3: omega 3 (sum of C18:3 c9,12,15 + C20:3 c11,14,17 + C20:4 c5,8,t10,c14 + C20:5 c8,11,14,17 + C22:5 c 4,7,10,13,16 + C22:6 n-3 c7,10,13,16,19)

⁶PUFA: polyunsaturated fatty acids (sum of n-6 + n-3).

In this study, feeding extruded linseed produced a significant decrease ($P < 0.01$) of the PUFA/SFA and n-6/n-3 ratios, with benefits for human health (Department of Health, 1994; Simopolous, 2001). In particular, the PUFA/SFA ratio should be greater than 0.4 and in our study the linseed diet increased this value to from 0.3 to 0.7. Furthermore, the n-6/n-3 ratio of 3.55 is below the recommended value of 4-5. Also other Authors found a decrease of the n-6/n-3 ratio in pig meat following a 5% flaxseed dietary addition and reported values similar to our results in other pig breeds (Matthews *et al.*, 2000; Juárez *et al.*, 2011; De Tonnac *et al.*, 2017).

Because of the higher content of PUFA and the lower content of C18:0, meat from extruded linseed fed pigs had lower ($P < 0.01$) atherogenicity and thrombogenicity indices. These are two important indicators related to human health since they indicate risks of increase of serum lipid and excess of platelet activity, respectively (Ulbricht & Southgate, 1991). Okrouhlá *et al.* (2013) found a significant decrease of the

thrombogenicity index in both barrows and gilts fed with linseed but did not report any dietary effect on the atherogenicity index.

Conclusions

A diet that includes 3% extruded linseed may present a viable option for feeding Nero Lucano pigs with few contraindications. Additional research should be carried out in order to assess whether the increase of the percentage of dietary linseed supplementation may further improve performance and meat quality in the autochthonous Nero Lucano pig breed.

Authors' Contributions

ST conceived and designed the study. MAC helped in designing the study, carrying out statistical analysis and interpretation of the data, collaborated in performing the experimental work, laboratory analysis, preparation of the manuscript and wrote the paper. ST and FG collaborated in animal management, in the collection of the productive performance data and carried out meat analysis. MR and AL collaborated in the preparation of the manuscript. AMF was involved in the statistics and interpretation of the data and constructive revision of the manuscript and helped with writing the paper.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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