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EFFECT OF COMBINED RAPESEED OIL AND GROUND FLAXSEED SUPPLEMENTATION ON FATTY ACID PROFILE, CHEMICAL COMPOSITION, AND LIPID HEALTH INDICES OF PORK

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Abstract

The present study investigated the effects of combined dietary supplementation with rapeseed oil (RO) and ground flaxseed (GF) on the fatty acid profile, chemical composition, and lipid nutritional health indices of m. Longissimus dorsi in Landrace × Large White finishing pigs. A total of 90 pigs were assigned to four dietary treatments – LC (control, no supplementation), L1 (1% RO + 1% GF), L2 (1% RO + 1.5% GF), and L3 (2% RO + 2% GF) – monitored across two production phases: Phase I (growing, 25-60 kg) and Phase II (finishing, 60–110 kg) over 84 days. Muscle samples from m. Longissimus dorsi were analysed for chemical composition (dry matter, crude protein, intramuscular fat, ash), complete fatty acid profile by gas chromatography–FID (GC-FID), and key lipid health indices: n-6/n-3 ratio, PUFA/SFA ratio, atherogenic index (AI), thrombogenic index (TI), peroxidizability index (PI), and hypocholesterolemic/hypercholesterolemic ratio (h/H). Alpha-linolenic acid (ALA, C18:3n-3) increased 5.8-fold in L3 (3.85%) vs. LC (0.62%, $p < 0.001$). The n-6/n-3 ratio decreased from 23.4:1 to 3.6:1 in L3 ($p < 0.001$), meeting the WHO (2002, 2023) recommendation. AI and TI were significantly reduced in L2 and L3 ($p < 0.001$). Crude protein and pH were unaffected. These findings confirm that combined RO + GF supplementation effectively transforms pork into a functional food with a markedly improved cardiovascular health profile.

Key words: *atherogenic index, fatty acid profile, flaxseed, functional pork, pigs, n-6/n-3 ratio, rapeseed oil, thrombogenic index.*

INTRODUCTION

The nutritional value of pork has become a central focus in both food science research and public health policy. Beyond traditional technological quality criteria such as pH, water holding capacity, and meat colour, consumers and nutritionists increasingly demand a favourable lipid composition, particularly an optimal balance between n-6 and n-3 polyunsaturated fatty acids (PUFA). The n-6/n-3 PUFA ratio in pork derived from conventional corn–soybean meal diets typically exceeds 15:1 to 22:1, far above the maximum of 4:1 recommended by the World Health Organization (WHO, 2002) and the European Food Safety Authority (EFSA, 2010). This imbalance contributes to pro-inflammatory dietary conditions associated with cardiovascular disease, type-2 diabetes, and metabolic syndrome (Wood et al., 2008).

Rapeseed oil (*Brassica napus* L.) and ground flaxseed (*Linum usitatissimum* L.) represent the most accessible and economically feasible plant-derived sources of n-3 PUFA in Central and Eastern European swine production. Rapeseed oil provides oleic acid (~60%), linoleic acid (~21%), and alpha-linolenic acid (ALA, ~9.5%), and carries the lowest atherogenic index (AI = 0.05) among common plant lipids (Khalili Tilami & Kouřimská, 2022). Ground flaxseed is one of the richest conventional plant sources of α-linolenic acid (ALA, 18:3n-3), which typically accounts for approximately 53–57% of its total fatty acid content. Dietary inclusion of flaxseed in pig diets has been consistently shown to increase ALA deposition in muscle and adipose tissue

and to reduce the n-6/n-3 PUFA ratio in pork toward nutritionally recommended levels (Czech et al., 2023).

Recent experimental evidence has confirmed that dietary flaxseed inclusion at 5-10% significantly increases ALA and total n-3 PUFA in the m. *Longissimus dorsi* of finishing pigs, while reducing the n-6/n-3 ratio to values approaching nutritional recommendations (Bartkovsky et al., 2022). Similarly, rapeseed oil supplementation for 4-6 weeks prior to slaughter significantly decreases the n-6/n-3 PUFA ratio in pig backfat and reduces atherogenic and thrombogenic indices (Okrouhla et al., 2018). For large and medium-heavy pig breeds, Minelli et al. (2023) demonstrated that lowering the dietary n-6/n-3 ratio from 9.7:1 to 1.4:1 in Large White pigs significantly increased n-3 PUFA in the Longissimus thoracis and reduced lipid peroxidation indices. However, the synergistic effect of combining rapeseed oil and ground flaxseed across a two-phase growing-finishing system and its impact on the full spectrum of lipid health indices has not been systematically studied.

The present study aimed to: formulate and characterise iso-energetic, iso-nitrogenous experimental diets incorporating graded levels of rapeseed oil and ground flaxseed for both growing and finishing phases; determine the effect of these diets on the chemical composition and complete fatty acid profile of m. *Longissimus dorsi*; calculate and interpret lipid health indices to assess the potential of dietary manipulation in producing functional pork with improved cardiovascular value.

MATERIALS AND METHODS

Animals, housing, and experimental design

The experiment was conducted over 84 consecutive days. A total of 90 clinically healthy Landrace × Large White pigs were used, with an initial mean body weight of 27.40 ± 0.82 kg. Animals were randomly allocated to four dietary treatment groups (n = 22-23 per group), each with three replicate pens of 7-8 animals: LC – control (0.95% sunflower oil in Phase I; 0.55% in Phase II, added for iso-energetic equalisation; no n-3 PUFA sources); L1 – 1% rapeseed oil (RO) + 1% ground flaxseed (GF); L2 – 1% RO + 1.5% GF; L3 – 2% RO + 2% GF. The experimental design was a completely randomised block with dietary treatment as the main factor.

Animals were housed in climate-controlled pens (18-22°C, relative humidity 60–70%, 16 h light) with ad libitum access to feed and water. Animal welfare procedures complied with Directive 2010/63/EU and Romanian Law No. 43/2014. Two complete compound feed formulations were prepared for each treatment group corresponding to the two production phases.

Experimental diets – formulation and nutritional characterisation

All diets were formulated to be approximately iso-energetic and iso-nitrogenous within each phase, meeting or exceeding NRC (2012) requirements for growing-finishing pigs. The base ingredients were corn grain, wheat grain, barley grain, soybean meal (44% CP), and wheat bran. To minimise metabolisable energy differences between groups arising from the lipid additions, the control diet (LC) was supplemented with refined sunflower oil (0.95% in Phase I; 0.55% in Phase II), replacing an equal proportion of corn grain. Sunflower oil was chosen for LC because it is essentially devoid of ALA (<0.5%) and does not contribute to n-3 PUFA enrichment of the meat, thus serving as an energetically equivalent but nutritionally neutral lipid vehicle. Rapeseed oil and sunflower oil were added by uniform spraying over the dry blend at a maximum mixing temperature of 40°C. Flaxseed was freshly milled to a particle size below 2 mm immediately before mixing to minimise ALA oxidation. Vitamin E was included in the premix at 150 mg/kg for all groups and 220 mg/kg for group L3, while BHT (125 mg/kg) was added to all groups as an antioxidant. Nutrient values were calculated using analysed and INRA (2004) feed composition tables.

The composition of the experimental diets and their calculated nutritional values are presented in Tables 1-4.

Table 1. Ingredient composition of experimental diets – Phase I: Growing (25-60 kg), % as-fed

Ingredients	LC (%)	L1 (%)	L2 (%)	L3 (%)
Lipid supplementation level	0% RO+0% GF	1% RO+1% GF	1% RO+1.5% GF	2% RO+2% GF
Corn grain	62.10	62.85	62.95	62.55
Wheat grain	8.00	7.00	7.00	6.50
Barley grain	6.50	5.50	5.00	4.50
Soybean meal (44% CP)	17.00	17.20	17.30	17.50
Wheat bran	1.00	1.00	0.80	0.50
Rapeseed oil	-	**1.00**	**1.00**	**2.00**
Ground flaxseed	-	**1.00**	**1.50**	**2.00**
Sunflower oil	0.95	-	-	-
Dicalcium phosphate	1.30	1.30	1.30	1.30
Calcium carbonate	1.40	1.40	1.40	1.40
Iodised salt (NaCl)	0.40	0.40	0.40	0.40
L-Lysine HCl (78%)	0.25	0.25	0.25	0.25
DL-Methionine (99%)	0.05	0.05	0.05	0.05
L-Threonine (98%)	0.05	0.05	0.05	0.05
Vitamin-mineral premix 1%	1.00	1.00	1.00	1.00
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**

LC = control group; L1, L2, L3 = experimental groups. RO = rapeseed oil; GF = ground flaxseed.

Table 2. Nutritional value of experimental diets – Phase I: Growing (25-60 kg)

Nutritional parameter	LC (%)	L1 (%)	L2 (%)	L3 (%)	NRC 2012 requirement
Metabolisable energy (MJ/kg)	13.65	13.66	13.70	13.97	13.40–13.80
Crude protein (%)	17.8	17.9	17.9	18.0	17.5–19.0
Crude fat (%)	4.40	5.05	5.60	7.35	-
Crude fibre (%)	2.85	2.80	2.78	2.70	max. 5.0
Lysine (%)	0.97	0.97	0.97	0.98	≥ 0.95
Met+Cys (%)	0.56	0.56	0.56	0.57	≥ 0.55
Threonine (%)	0.62	0.62	0.62	0.63	≥ 0.61
Total Calcium (%)	0.72	0.72	0.72	0.72	0.70–0.80
Total Phosphorus (%)	0.56	0.56	0.56	0.55	0.55–0.65
Linoleic acid LA (C18:2n-6, %)	2.08	2.05	2.12	2.60	-
α-Linolenic acid ALA (C18:3n-3, %)	**0.10**	**0.41**	**0.68**	**1.20**	-
n-6/n-3 PUFA ratio	**20.8:1**	**5.0:1**	**3.1:1**	**2.2:1**	≤ 10:1

Refined rapeseed oil, erucic acid < 2%. Ground flaxseed, milled to < 2 mm particle size immediately before mixing. Premix provided per kg complete feed: vitamin A 8,000 IU; vitamin D_3 1,200 IU; vitamin E 150 mg (LC, L1, L2) / 220 mg (L3); vitamin K_3 2 mg; B-complex vitamins; Fe 100 mg; Cu 10 mg; Zn 80 mg; Mn 40 mg; I 0.3 mg; Se 0.3 mg; Co 0.2 mg; phytase ≥ 500 FTU.

Table 3. Ingredient composition of experimental diets – Phase II: Finishing (60-110 kg), % as-fed

Ingredients	LC (%)	L1 (%)	L2 (%)	L3 (%)
Corn grain	66.90	66.45	66.55	66.15
Wheat grain	7.00	6.50	6.50	6.00
Barley grain	6.00	5.50	5.00	4.50
Soybean meal (44% CP)	13.00	13.30	13.40	13.50
Wheat bran	2.50	2.20	2.00	1.80
Rapeseed oil	-	**1.00**	**1.00**	**2.00**
Ground flaxseed	-	**1.00**	**1.50**	**2.00**
Sunflower oil	0.55	-	-	-
Dicalcium phosphate	1.10	1.10	1.10	1.10
Calcium carbonate	1.30	1.30	1.30	1.30
Iodised NaCl	0.40	0.40	0.40	0.40
L-Lysine HCl (78%)	0.20	0.20	0.20	0.20
DL-Methionine (99%)	0.03	0.03	0.03	0.03
L-Threonine (98%)	0.02	0.02	0.02	0.02
Vitamin-mineral premix	1.00	1.00	1.00	1.00
TOTAL (%)	**100.00**	**100.00**	**100.00**	**100.00**

LC = control group; L1, L2, L3 = experimental groups. RO = rapeseed oil; GF = ground flaxseed.

Table 4. Nutritional value of experimental diets – Phase I: Growing (25-60 kg)

Nutritional parameter	LC (%)	L1 (%)	L2 (%)	L3 (%)	NRC 2012 requirement
Metabolisable energy (MJ/kg)	13.69	13.81	13.85	14.12	13.20–13.60
Crude protein (%)	15.6	15.7	15.8	15.9	15.5–17.0
Crude fat (%)	3.86	5.00	5.55	7.28	-
Crude fibre (%)	3.20	3.15	3.12	3.05	max. 5.0
Lysine (%)	0.76	0.76	0.77	0.77	≥ 0.75
Met+Cys (%)	0.45	0.45	0.45	0.46	≥ 0.44
Threonine (%)	0.50	0.50	0.50	0.51	≥ 0.49
Total Calcium (%)	0.63	0.63	0.63	0.63	0.60–0.70
Total Phosphorus (%)	0.50	0.50	0.50	0.49	0.45–0.55
Linoleic acid LA (C18:2n-6, %)	1.82	1.98	2.06	2.55	-
α-Linolenic acid ALA (C18:3n-3, %)	**0.08**	**0.38**	**0.65**	**1.17**	-
n-6/n-3 PUFA ratio	**22.8:1**	**5.2:1**	**3.2:1**	**2.2:1**	≤ 10:1

Refined rapeseed oil, erucic acid < 2%. Ground flaxseed, milled to < 2 mm particle size immediately before mixing. Premix provided per kg complete feed: vitamin A 8,000 IU; vitamin D_3 1,200 IU; vitamin E 150 mg (LC, L1, L2) / 220 mg (L3); vitamin K_3 2 mg; B-complex vitamins; Fe 100 mg; Cu 10 mg; Zn 80 mg; Mn 40 mg; I 0.3 mg; Se 0.3 mg; Co 0.2 mg; phytase ≥ 500 FTU.

The dietary n-6/n-3 ratio decreased progressively from 20.3:1 (Phase I) and 22.1:1 (Phase II) in LC to 2.2:1 in L3 in both phases, driven by the combined ALA contribution of rapeseed oil (~9.5% ALA) and ground flaxseed (~57% ALA of total fat). This gradient ensures a clear dose-response relationship between dietary treatment and the n-3 PUFA status of muscle tissue.

Sample collection and analytical methods

At slaughter (day 84, live weight 106-109 kg), all 90 pigs were processed at a commercial abattoir. Samples of m. *Longissimus dorsi* were collected bilaterally at the level of the last rib (≈500 g per animal), vacuum-packed immediately, and stored at −20°C until analysis. Chemical composition was determined following AOAC (2019) methods: dry matter (AOAC 950.46), crude protein by Kjeldahl × 6.25 (AOAC 981.10), intramuscular fat by Soxhlet extraction (AOAC 991.36), and ash by incineration at 550°C (AOAC 942.05). Muscle pH was measured at 45 min (pH_{45}) and 24 h (pH_{24}) post-mortem using a portable pH meter (Testo 205) inserted directly into the *Longissimus dorsi*.

Fatty acid methyl esters (FAMEs) were prepared by Folch extraction followed by base-catalysed transesterification with KOH/methanol (0.5 M) and methylation with BF_3/methanol (14%). FAMEs were separated and quantified on a Shimadzu GC-2010 Plus system (FID detector; SP-2560 capillary column, 100 m × 0.25 mm × 0.2 µm). Results are expressed as g/100g total fatty acids. Individual fatty acids were identified using the Supelco 37 FAME Mix standard.

Lipid Nutritional Health Indices

The following indices were calculated from the fatty acid profile of intramuscular fat:

n-6/n-3 ratio = Σ n-6 PUFA / Σ n-3 PUFA

PUFA/SFA ratio = Σ PUFA / Σ SFA

Atherogenic Index (AI) = [C12:0 + 4×C14:0 + C16:0] / [Σ MUFA + Σ PUFA] (Ulbricht & Southgate, 1991)

Thrombogenic Index (TI) = [C14:0 + C16:0 + C18:0] / [0.5×ΣMUFA + 0.5×Σn-6 PUFA + 3×Σn-3 PUFA + (n-3/n-6)] (Ulbricht & Southgate, 1991)

Peroxidizability Index (PI) = 0.025×%MUFA + 1×%C18:2n-6 + 2×%C18:3n-3 + 4×%C20:4n-6 + 8×%C20:5n-3 + 11×%C22:5n-3 + 12×%C22:6n-3 (Erickson, 1993)

h/H (hypo/hypercholesterolemic) ratio = (C18:1n-9 + Σ PUFA) / (C14:0 + C16:0) (Santos-Silva et al., 2002)

Statistical Analysis

Data were analysed by one-way ANOVA in SPSS v.26 (IBM Corp.), with treatment group as the fixed effect. Means were compared using Tukey's HSD post-hoc test ($\alpha = 0.05$). Normality was verified by the Shapiro-Wilk test. Results are expressed as means ± SEM.

RESULTS AND DISCUSSIONS

Chemical composition of m. *Longissimus dorsi*

The chemical composition data are presented in Table 5. No significant differences were found between treatments for dry matter ($p = 0.312$), crude protein ($p = 0.845$), or ash content ($p =$

0.920), indicating that the dietary modifications did not alter the fundamental structural composition of muscle tissue. This is consistent with the meta-analytical findings of Wang et al. (2021), who confirmed across 14 studies (752 pigs) that PUFA supplementation does not significantly affect the crude protein content of the *Longissimus dorsi* ($p > 0.05$).

Table 5. Chemical composition of m. *Longissimus dorsi* (%, fresh weight; means ± SEM, n = 22–23/group)

Parameter	LC	L1	L2	L3
Dry matter (%)	26.15±0.28	25.92±0.31	25.78±0.26	25.65±0.33
Crude protein (%)	22.48±0.22	22.55±0.25	22.52±0.21	22.60±0.26
Intramuscular fat – IMF (%)	**1.92±0.12^{a}**	**1.98±0.14^{a}**	**2.10±0.11^{ab}**	**2.28±0.15^{b}**
Ash (%)	1.14±0.02	1.15±0.02	1.15±0.02	1.16±0.02
pH_{45} (45 min post-mortem)	6.42±0.08	6.45±0.09	6.48±0.08	6.50±0.10
pH_{24} (24 h post-mortem)	5.62±0.05	5.65±0.06	5.68±0.05	5.72±0.06

Different superscript letters within a row indicate significant differences ($p < 0.05$, Tukey HSD). IMF = intramuscular fat determined by Soxhlet extraction. pH_{45} and pH_{24} measured directly in m. *Longissimus dorsi*

Intramuscular fat (IMF) was significantly higher in L3 (2.28%) compared to LC (1.92%, $p = 0.032$). This moderate increase aligns with the meta-analytical weighted mean difference of +0.467% reported by Wang et al. (2021) for PUFA-supplemented pigs, and is likely attributable to stimulated hepatic lipogenesis from elevated dietary lipid intake in L3 (4% total added fat). Crude protein content was statistically equivalent across all groups (22.48–22.60%, $p = 0.845$), confirming that protein deposition efficiency was not impaired by the supplementation. pH values fell within the physiological norm (pH_{45}: 6.42–6.50; pH_{24}: 5.62–5.72), excluding PSE or DFD anomalies. Jankowiak et al. (2021) established that pH_{45} values above 6.30 are associated with higher water holding capacity and lower drip loss, a condition satisfied by all treatment groups in this study.

Fatty acid profile of intramuscular fat

The complete fatty acid profile of intramuscular fat from m. Longissimus dorsi is presented in Table 6. Dietary supplementation produced highly significant modifications in the PUFA fraction while exerting comparatively modest effects on SFA and MUFA content.

Table 6. Fatty acid profile of m. *Longissimus dorsi* intramuscular fat
(g/100 g total fatty acids; means ± SEM, n = 22-23/group)

Fatty acid	LC	L1	L2	L3
Saturated Fatty Acids (SFA)				
C14:0 (Myristic)	1.52±0.08	1.48±0.09	1.44±0.08	1.38±0.10
C16:0 (Palmitic)	24.80±0.42^{a}	24.15±0.48^{ab}	23.50±0.40^{b}	22.80±0.52^{c}
C18:0 (Stearic)	12.35±0.38	12.10±0.42	11.85±0.36	11.55±0.45
Σ SFA	**38.67±0.65^{a}**	**37.73±0.72^{ab}**	**36.79±0.62^{b}**	**35.73±0.78^{c}**
Monounsaturated Fatty Acids (MUFA)				
C16:1n-7 (Palmitoleic)	2.85±0.18	2.80±0.20	2.75±0.17	2.68±0.22
C18:1n-9 (Oleic)	40.55±0.88^{a}	41.25±0.95^{a}	42.10±0.85^{b}	43.40±1.02^{c}
Σ MUFA	**43.40±0.92^{a}**	**44.05±1.02^{a}**	**44.85±0.90^{b}**	**46.08±1.08^{c}**
n-6 Polyunsaturated Fatty Acids				
C18:2n-6 (Linoleic – LA)	14.50±0.35	14.25±0.38	14.10±0.33	13.85±0.40
C20:4n-6 (Arachidonic)	0.82±0.05	0.80±0.06	0.78±0.05	0.75±0.06
Σ n-6 PUFA	**15.32±0.38**	**15.05±0.42**	**14.88±0.36**	**14.60±0.44**
n-3 Polyunsaturated Fatty Acids				
C18:3n-3 (ALA – α-Linolenic)	0.62±0.06^{a}	1.82±0.14^{b}	2.68±0.20^{c}	3.85±0.28^{d}
C20:5n-3 (EPA)	0.05±0.01^{a}	0.12±0.02^{b}	0.17±0.02^{c}	0.24±0.03^{d}
C22:5n-3 (DPA)	0.28±0.03^{a}	0.42±0.04^{b}	0.55±0.04^{c}	0.72±0.06^{d}
Σ n-3 PUFA	**0.95±0.08^{a}**	**2.36±0.18^{b}**	**3.40±0.24^{c}**	**4.81±0.34^{d}**
PUFA Summary				
Σ PUFA (total)	**16.27±0.42^{a}**	**17.41±0.52^{b}**	**18.28±0.48^{c}**	**19.41±0.62^{d}**
PUFA/SFA ratio	**0.421±0.012^{a}**	**0.461±0.015^{ab}**	**0.497±0.014^{b}**	**0.543±0.018^{c}**

Different superscript letters (a,b,c,d) within a row indicate significant differences ($p < 0.05$, Tukey HSD). Green-highlighted section: n-3 PUFA fraction with direct experimental relevance. ALA = alpha-linolenic acid (C18:3n-3); EPA = eicosapentaenoic acid (C20:5n-3); DPA = docosapentaenoic acid (C22:5n-3).

The most significant outcome was the highly significant, dose-dependent increase in ALA content: from 0.62% in LC to 1.82% in L1, 2.68% in L2, and 3.85% in L3 ($p < 0.001$), representing a 5.8-fold enrichment in L3 vs. control. This linear dose-response pattern is consistent with the findings of Bartkovsky et al. (2022), who reported statistically significant ALA accumulation in m. *Longissimus dorsi* of pigs fed 5% and 10% flaxseed for 3-6 weeks, with the effect proportional to both inclusion level and duration. The direct dietary incorporation of ALA into muscle phospholipids in monogastric species is well established, as dietary fatty acids absorbed from the intestine are incorporated into tissue lipids largely unchanged (Okrouhla et al., 2018).

EPA (C20:5n-3) and DPA (C22:5n-3) also increased significantly across all supplemented groups ($p < 0.001$), indicating active, albeit limited, elongase and desaturase activity in the Landrace × Large White genotype. Minelli et al. (2023) similarly observed significant increases in n-3 long-chain PUFA in the *Longissimus thoracis* of Large White pigs fed a low n-6/n-3 diet, with the magnitude of transfer from diet to muscle being consistent with the 35-50% efficiency commonly reported for ALA incorporation in monogastric tissues.

Total SFA decreased significantly from 38.67% in LC to 35.73% in L3 ($p = 0.004$), driven primarily by a reduction in palmitic acid (C16:0, $p = 0.028$). This is attributed to the known inhibitory effect of elevated dietary PUFA on the activity of stearoyl-CoA desaturase (SCD) and de novo fatty acid synthesis in the liver. MUFA showed a significant increase, particularly oleic acid (C18:1n-9: 40.55% in LC vs. 43.40% in L3, $p = 0.045$), reflecting the high oleic acid content of rapeseed oil (~60% C18:1) incorporated into the diet. The PUFA/SFA ratio improved progressively from 0.421 in LC to 0.543 in L3 ($p < 0.001$), with the recommended threshold of 0.45 being surpassed from group L2 onward.

Lipid Nutritional Health Indices

The lipid health indices calculated from intramuscular fatty acid profiles are presented in Table 7.

Table 7. Lipid nutritional health indices of m. *Longissimus dorsi* intramuscular fat (means ± SEM, n = 22-23/group)

Lipid health index	LC	L1	L2	L3
n-6/n-3 PUFA ratio	23.4±1.8^{a}	6.4±0.5^{b}	4.4±0.4^{c}	3.6±0.3^{c}
PUFA/SFA ratio	0.421±0.012^{a}	0.461±0.015^{ab}	0.497±0.014^{b}	0.543±0.018^{c}
Atherogenic Index (AI)	0.612±0.022^{a}	0.568±0.024^{ab}	0.528±0.020^{b}	0.482±0.025^{c}
Thrombogenic Index (TI)	1.245±0.048^{a}	1.118±0.052^{ab}	1.012±0.044^{b}	0.895±0.055^{c}
Peroxidizability Index (PI)	78.5±3.2^{a}	94.8±4.1^{b}	108.2±3.8^{c}	128.5±5.2^{d}
h/H ratio	2.08±0.08^{a}	2.18±0.09^{a}	2.28±0.08^{b}	2.42±0.10^{c}
Σ n-3 PUFA (% total FA)	0.95±0.08^{a}	2.36±0.18^{b}	3.40±0.24^{c}	4.81±0.34^{d}

Different superscript letters within a row indicate significant differences ($p < 0.05$). AI = atherogenic index; TI = thrombogenic index; PI = peroxidizability index; h/H = hypocholesterolemic/hypercholesterolemic ratio.

The n-6/n-3 PUFA ratio was dramatically reduced in all supplemented groups: from 23.4:1 in LC to 6.4:1 in L1, 4.4:1 in L2, and 3.6:1 in L3 ($p < 0.001$). Groups L2 and L3 achieve or surpass the WHO (2002) recommendation of ≤ 4:1. For comparison, Minelli et al. (2023) achieved a muscle n-6/n-3 ratio of approximately 2.8:1 in Large White pigs fed a dietary ratio of 1.4:1, a more extreme intervention. In our system, the dietary ratio of 2.2:1 in L3 translates to a muscle ratio of 3.6:1, suggesting an approximately 39% transfer efficiency, in agreement with published values for ALA incorporation in monogastric muscle.

The atherogenic index (AI) was significantly reduced in L2 (0.528) and L3 (0.482) compared to LC (0.612; $p < 0.001$), in line with the known effect of rapeseed oil, which carries one of the lowest AI values among common plant lipids (AI = 0.05; Khalili Tilami & Kouřimská, 2022). The thrombogenic index (TI) decreased from 1.245 in LC to 0.895 in L3 ($p < 0.001$). Okrouhla et al. (2018) reported analogous significant reductions in both AI and TI in the backfat of DanBred pigs fed 40 g/kg rapeseed oil for 4–6 weeks, supporting the consistency of our findings across species, genotypes, and tissue types.

The peroxidizability index (PI) increased significantly with supplementation (78.5 in LC to 128.5 in L3, $p < 0.001$), reflecting the intrinsic oxidative susceptibility of n-3 PUFA-enriched

fat. Sobeková et al. (2023) found that 6 weeks of flaxseed supplementation significantly increased TBARS content in cardiac and brain tissue of fattening pigs ($p < 0.05$), underscoring the need for adequate antioxidant protection. In the present experiment, vitamin E was increased to 220 mg/kg NC in L3 and ethoxyquin was included in the premix of all supplemented groups; however, TBARS analysis of meat samples was not performed and is recommended for future investigations. The h/H ratio improved significantly in L2 (2.28) and L3 (2.42) vs. LC (2.08; $p = 0.002$), reflecting the combined contribution of increased oleic acid (from RO) and n-3 PUFA (from GF) to the overall cholesterol-lowering fatty acid profile.

CONCLUSIONS

The combined supplementation with rapeseed oil and ground flaxseed produced a highly significant, dose-dependent enrichment of m. Longissimus dorsi intramuscular fat with ALA (5.8-fold increase in L3 vs. LC; $p < 0.001$) and its elongation products EPA and DPA. Total n-3 PUFA increased from 0.95% to 4.81% of total fatty acids in L3. These results confirm that the Landrace × Large White hybrid possesses the metabolic capacity to incorporate dietary n-3 PUFA into muscle lipids proportionally to dietary supply.

All lipid health indices were significantly improved by the supplementation. The n-6/n-3 ratio was reduced from 23.4:1 to 3.6:1 in L3, meeting the WHO (2023) recommendation of ≤ 4:1 from group L2 onward. The AI decreased from 0.612 to 0.482 and the TI from 1.245 to 0.895 in L3 ($p < 0.001$). The h/H ratio and PUFA/SFA ratio were improved significantly in L2 and L3. Pork from L2 and L3 can be considered a functional food with a demonstrably improved cardiovascular nutritional profile.

The combination of 1% rapeseed oil + 1.5% ground flaxseed (L2) is recommended as the optimal practical level for commercial applications: it achieves an n-6/n-3 ratio of 4.4:1, surpasses the PUFA/SFA threshold of 0.45, significantly reduces AI and TI, and maintains a PI (108.2) below the critical threshold for unacceptable oxidative instability, without requiring extraordinary antioxidant interventions. The L3 combination (2% RO + 2% GF) achieves superior n-3 enrichment but necessitates strict antioxidant management (vitamin E ≥ 220 mg/kg NC, ethoxyquin ≥ 125 ppm) to mitigate oxidative risks during storage.

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