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Dietary supplementation with vegetable oils with low n-6:n-3 polyunsaturated fatty acid ratios improves the intramuscular fat and fatty acid composition of growing-finishing pigs

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Abstract

This study investigated the effects of different n-6:n-3 polyunsaturated fatty acid (PUFA) ratios in pig diets using vegetable oil supplementation on growth performance, carcass characteristics, meat quality, and fatty acid composition in the longissimus dorsi muscle (LM) and subcutaneous adipose tissue (SAT) of pigs. Fifty-four cross-bred growing pigs (Large White × Landrace × Duroc; 45.03 ± 1.72 kg) were randomly assigned to one of three isoenergetic diets: the 1) basal diet (control), 2) RAPO diet (diet supplemented with 4.5% rapeseed oil), and 3) MIXO diet (diet supplemented with 2.25% rapeseed oil and 2.25% linseed oil), with n-6:n-3 PUFA ratios of approximately 13:1, 7:1, and 2:1, respectively. The experiment lasted 42 days. The RAPO and MIXO diets did not affect the growth performance and carcass characteristics of pigs, while the content of low-density lipoprotein (LDL), triglycerides (TGs), and glucose (GLU) in the serum decreased substantially. Intramuscular fat (IMF) content increased by 19.25% and 20.11% in the LM of pigs fed the RAPO and MIXO diets, respectively, and lower cooking loss and drip loss of meat were observed only in pigs fed the MIXO diet. The RAPO and MIXO diets decreased the stearic acid and palmitoleic acid levels, increased the total PUFA levels (including those of α-linolenic and linoleic acid), and decreased the n-6:n-3 PUFA ratios in LM and SAT. However, the MIXO diet was more effective in improving the fatty acid composition. A mixed oil diet with an n-6:n-3 PUFA ratio of 2:1 is an effective measure for improving meat quality.

Keywords: meat quality, growth performance, intramuscular fat, fatty acids, pig
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Introduction

Fat mass and distribution are essential factors determining the economic traits of pigs. Reduction in subcutaneous fat content increases carcass value, and intramuscular fat (IMF) is known for “marbling” and is positively associated with various meat quality traits, such as juiciness, flavour, and tenderness (Dannenberger *et al*., 2012). Previous studies have proposed that a minimum level of 2.5% IMF is needed to positively influence the eating quality and sensory quality traits, thereby increasing consumer acceptance of pork (Alfaia *et al*., 2019). In addition, the type and content of fatty acids in pork have received much attention due to the nutritional value and health benefits of fatty acids in the human diet, especially unsaturated fatty acids (Puig-Oliveras *et al*., 2014). However, genetic selection for increased meat yield has resulted in a less than 1.5% IMF content in most modern pig breeds and has altered the fatty acid composition of meat (Hernández-Sánchez *et al*., 2013; Ba *et al*., 2019), and typical corn–soybean meal-based diets lead to a high ratio of n-6:n-3 PUFA in pork (Palmquist, 2009). A high n-6:n-3 PUFA ratio in the human diet could be a factor inducing cardiovascular, inflammatory, and metabolic disorders (Martínez-Fernández *et al*., 2015). There is growing interest in producing pork with a high IMF content, balanced fatty acid composition, and low subcutaneous fat content (Alfaia *et al*., 2019).

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Dietary fat sources and their n-6:n-3 PUFA ratios play an essential role in altering the fatty acid composition in tissues, maintaining healthy lipid metabolism, and promoting animal production performance. Studies have focused on altering the fatty acid composition of pork by supplementing the diet with natural sources of PUFAs, such as fish oil or fish meal, different linseed products, rapeseed oil, and soybean oil (Realini *et al*., 2010; Okrouhla *et al*., 2018), which could be suitable fat sources for enriching n-3 PUFAs in animal products. For example, the inclusion of 3% extruded linseed or linseed oil in the diet improved the fatty acid profile of pork without affecting growth performance (Hăbeanu *et al*., 2014; Tarricone *et al*., 2020). However, different vegetable oils or n-6:n-3 PUFA ratios in diets had inconsistent effects on the IMF content and fatty acid composition of pigs. A meta-analysis showed that dietary linseed supplementation increased the levels of n-3 PUFAs, such as α-linolenic acid, docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), in muscle and adipose tissue (Corino *et al*., 2014). Okrouhlá *et al*. (2018) reported that rapeseed oil was better than soybean oil in improving the fatty acid composition and reducing the n-6:n-3 PUFA ratio in subcutaneous fat. Dietary n-6:n-3 PUFA ratios of 4:1 and 2:1 substantially increased the proportions of essential fatty acids and reduced the cooking loss ratio, but low expression of acetyl COA carboxylase (ACC) with a dietary n-6:n-3 PUFA ratio of 2:1 indicated a reduced capacity for *de novo* fatty acid synthesis (Song *et al*., 2020). Palmitic, oleic, and linoleic acids, as dietary fat sources, have been confirmed to promote lipid accumulation in porcine adipocytes through different metabolic pathways, which could be related to the phosphorylation of peroxisome proliferator-activated receptor γ (PPARγ) (Yu *et al*., 2017). Moreover, an appropriate n-6:n-3 PUFA ratio in the diet is beneficial in improving lipid metabolism and the inflammatory system to ensure the efficient utilization of energy and nutrients (Duan *et al*., 2014). Given the role of dietary n-6 and n-3 PUFAs and their ratio in regulating fat metabolism and health benefits, their impact on the improvement of meat quality needs to be elucidated.

The purpose of this study was to investigate whether the addition of vegetable oils with different n-6:n-3 PUFA ratios to the diet as a partial carbohydrate replacement could improve the production performance, health status, and meat quality in finishing pigs; and to further clarify the effects of dietary n-6:n-3 PUFA ratios on fatty acid metabolism in longissimus dorsi muscle (LM) and subcutaneous adipose tissue (SAT).

Materials and Methods

This study was approved by the Animal Care and Use Committee of Northwest Minzu University (Lanzhou, China; ethical clearance number: 20180307). A total of 54 barrows (Large White × Landrace × Duroc) at approximately 13 weeks of age, with an initial body weight of 45.03 ± 1.72 kg, were used. The pigs were provided by Lanzhou Ruiyuan Agricultural Technology Co. Ltd (Lanzhou, China). Pigs were allotted randomly to three treatments, and each treatment had six replicates and three pigs per pen. Pigs were housed in an enclosed building with a partially-slatted concrete floor. Each pen provided 4.68 m^2 floor space with a size of 2.6 m × 1.8 m and was equipped with a stainless-steel feeder and nipple drinkers. Feed and fresh water were provided *ad libitum*. During the experimental period, the average temperature inside the building was 20–28 °C (average 24 °C), and the relative humidity was 56 ± 6%. All pigs were fed twice daily (at 08:00 and 17:00). Pigs were given a 7-day adaptation period before the experiment. The experiment lasted 42 days.

Each group was fed one of three isoenergetic diets: the control diet (high-carbohydrate diet), RAPO diet (diet supplemented with 4.5% rapeseed oil), and MIXO diet (diet supplemented with 2.25% rapeseed oil and 2.25% linseed oil) with an n-6:n-3 PUFA ratio of approximately 13:1, 7:1 and 2:1, respectively. Diets were formulated to meet the requirements proposed by the NRC (2012). The contents of digestible energy (DE), crude protein (CP), and acid detergent fibre (ADF) in the diets were approximately equal. Feed samples were taken from each dietary treatment for chemical analysis. The ingredients, chemical composition, and fatty acid composition of each diet are shown in Table 1.

Table 1 Ingredient and chemical composition of experimental diets (DM basis, %)

Item	Dietary treatment[1]		
	Control	RAPO	MIXO
Ingredients, %			
Corn	60.50	56.00	56.00
Soybean meal	23.00	22.00	22.00
Wheat bran	5.25	9.00	9.00
Premix[2]	4.00	4.00	4.00
NaCl	0.25	0.25	0.25
Rapeseed oil	0	4.50	2.25
Linseed oil	0	0	2.25
Alfalfa meal	0	4.25	4.25
Starch	7.00	0	0
Total	100.00	100.00	100.00
Chemical composition, %			
Digestive energy, MJ/kg	13.66	13.79	13.79
Crude protein	15.84	15.99	15.99
Ether extract	2.76	7.24	7.24
Crude fibre	3.68	4.07	4.07
Neutral detergent fibre	11.08	12.67	12.67
Acid detergent fibre	4.41	4.56	4.56
Calcium	0.72	0.75	0.75
Total phosphorus	0.45	0.46	0.46
Available phosphorus	0.23	0.23	0.23
Lysine	0.91	0.93	0.93
Methionine	0.23	0.23	0.23
Tryptophan	0.18	0.19	0.19
Fatty acid composition of diets, g/100g of total fatty acids			
Palmitic acid, C16:0	15.41	13.52	12.07
Stearic acid, C18:0	2.24	3.24	3.46
Oleic acid, C18:1	15.83	25.35	24.58
α-Linolenic acid, C18:3n-3	1.72	6.31	17.78
Linoleic acid, C18:2n-6	23.31	45.44	39.97
Arachidic acid, C20:0	0.24	0.42	0.46
n-6 PUFA	23.34	45.48	40.02
n-3 PUFA	1.73	6.32	17.79
n-6 PUFA: n-3 PUFA[3]	13.49	7.19	2.25

[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil
[2]One kg of premix provided the following: VA, 120 000 IU; VD_3, 16 000 IU; VE, 440 IU; VK_3, 80 mg; VB_1, 40 mg; VB_2, 120 mg; VB_6, 40 mg; VB_{12}, 0.40 mg; nicotinamide, 560 mg; pantothenic acid, 400 mg; biotin, 4.00 mg; folic acid, 24 mg; Fe, 2 000 mg; Cu, 640 mg; Zn, 1 600 mg; Mn, 320 mg; I, 9.60 mg; Se, 4.00 mg; lysine, 32 g; calcium, 150 g; total phosphorus, 21 g; and sodium chloride, 70 g.
[3]n-6: n-3 PUFA, [sum of n-6 PUFAs/sum of n-3 PUFAs] ratio

Feed consumption was recorded daily per pen. Data were collected to calculate the initial body weight, final body weight, average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR).

At the end of the experiment (42 days), all pigs were weighed individually before the morning feeding. Approximately 5 mL of blood was collected from the anterior vena cava of pigs, centrifuged at 2,000 × *g* for 5 min, and then frozen at -20 °C until the assay was conducted. Ten pigs with weights closest to the average weight of each group were chosen, for a total of 30 pigs. The pigs were electrically stunned (225 to 380 V/0.5

A for 5 to 6 s), slaughtered, scalded (4 min, 63 °C), dehaired, and eviscerated. Samples of the LM were taken from the 3rd to 11th rib on the left half of the carcasses to determine meat quality parameters. Samples of the LM and SAT were rapidly collected from the 13th to 14th ribs on the right side of the carcass, frozen in liquid nitrogen and stored at -80 °C until fatty acid composition analysis and gene expression analysis. Backfat thickness was measured on the right side of the carcass as the average value for three points: the shoulder, the last rib, and the last lumbar vertebra. Loin eye area was measured with the maximum width (cm) and height (cm) of the exposed surface muscle area between the 12th and 13th ribs on the right side of the carcass and was calculated using the equation:

Loin eye area (cm^2) = loin eye height (cm) × width (cm) × 0.7, (1)

according to the Chinese Guidelines on Performance Measurement Technology and Regulations for Pigs (China, 2014).

After 24 h of refrigeration at 4 °C, the meat colour was measured using a colorimeter (CR-400, Minolta Co., Osaka, Japan), and the average values of lightness (L*), redness (a*), and yellowness (b*) were determined from five readings. The pH values were measured with a pH meter (MP 220, Mettler-Toledo, Schwarzenbach, Switzerland). Drip loss was determined using the suspension method. To measure drip loss, the meat samples were trimmed to remove the peripheral muscular membranes and cut into blocks (approximately 2 × 2 × 2 cm^3) along the direction of muscle fibres. The meat pieces were suspended in plastic bags and stored at 4 °C for 24 h. The drip loss was calculated as a percentage of weight loss compared to the initial weight, using the average of three experiments. To evaluate the shear force, any visible external fat and connective tissue were removed from the samples, which were individually packed in plastic bags and heated in a water bath until the core temperature reached 70 °C. After the samples were cooled to room temperature (25 °C), each cooled sample was cut into three slices, parallel to the fibre orientation (1.27 cm in diameter and ~3 cm in length) and measured using a shear apparatus (C-LM3, Northeast Agricultural University, Harbin, China). After weighing, the samples were placed in plastic bags and boiled in water until the internal temperature reached 70 °C. Cooking loss was calculated as the percentage of weight loss after cooking. The water loss ratio was measured using a Model RH-1000 instrument (RH-1000, RunHu Co., Guangdong, China).

The samples were cut into a circular meat sample with an area of 5 cm^2 and a thickness of 1 cm, weighed and placed between gauze and 18 layers of qualitative filter paper. Then, the circular meat samples were pressurized to 35 kg (stress of 138.8 kPa) for 5 min and weighed. The water loss percentage was calculated as the fluid lost compared to the initial meat weight.

IMF content was determined in LM using the Soxhlet extraction method. The fatty acid composition was determined using gas chromatography, as previously described (Xu, *et al*., 2019). Briefly, lipids were extracted from samples using 2:1 chloroform:methanol according to the Folch method (Folch *et al*., 1957). Then, total lipids were converted into fatty-acid methyl esters (FAMEs) and analysed using gas chromatography (GC-2010 Plus, Shimadzu Co., Kyoto, Japan) equipped with a flame ionization detector (FID) and a SP2560 capillary column (100 mm × 0.25 mm, 0.2 μm). The results are expressed as a percentage of total fatty acids.

Serum levels of triglycerides (TGs), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose (GLU), serum creatinine (Scr), and blood urea nitrogen (BUN) were analysed using an automatic biochemical analyser (7100 Automatic Analyzer, Hitachi, Tokyo, Japan). Fasting serum insulin (INS) levels were analysed using an enzyme-linked immunosorbent assay (ELISA).

Total RNA was extracted from the LM and SAT samples using TRIzol reagent (Fermentas RNA Extraction Kit). RNA concentration and purity were evaluated by a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA). Reverse transcription was performed using the TaqMan Reverse Transcription Reagent. Real-time, quantitative polymerase chain reaction (qPCR) was performed using a CFX96 real-time PCR detection system (Bio–Rad, Hercules, CA, USA). The PCR system was set as follows (total reaction system: 10 μl): 5.0 μl of SYBR Premix Ex Taq™ (TaKara, Kyoto, Japan), 0.4 μl of each primer, 1.0 μl of cDNA solution, and 3.2 μl of sterile double-steamed water. The PCR conditions were 40 cycles at 95 °C for 30 s (denaturation), 60 °C for 30 s (annealing), and 72 °C for 30 s (extension). Relative quantification was performed with the $2^{-\Delta\Delta Ct}$ method. Primers were designed using Primer Premier 5.0 software (Table 2).

Table 2 Specific primers used for real-time quantitative PCR

Gene name[1]	Accession number	Primer sequence (5'-3')	Size (bp)
FAS	NM_001099930	F: AAGCAGGCGAACACGATG R: GAAGGGAAGCAGGGTTGATG	94
SCD1	XM_021072070	F: TGCTGATCCCCACAATTCCC R: CTCCCGGGGGCTAATAGTCT	352
Elovl5	XM_021098832	F: TGTGAGTTGTGCCCAATGCT R:	166
FABP4	NM_001002817	F: ATGGCCAAACCCAACCTGAT R: GGTGGTTGTCTTTCCATCCCA	143
ChREBP	DQ372586.1	F: GCTCAACGCTGCCATCAA R: GTCCCGCATCTGGTCAAAG	88
HSL	NM_214315	F: GGATATGCCTCGCAGGAGAC R: AAGTTGGGTCGGGACTTGTG	195
LPL	NM_214286	F: CGACGCAGATTTTGTAGACG R: CTCATGGGAGCACTTCACG	148
β-actin	XM_003124280	F: GATCGTGCGGGACATCAA R:	180

[1]FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase 1; Elovl5, fatty acid elongase-5; FABP4, fatty acid-binding protein 4; ChREBP, carbohydrate response element-binding protein; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase.

Data were analysed using one-way analysis of variance (ANOVA); the Duncan method was used to test multiple comparisons using SPSS statistical software (SPSS Inc., Chicago, IL, USA). The data in the tables are presented as the means and standard error of the mean (SEM). Results were considered significant when the *P* value was <0.05, and *P* values within 0.05 and 0.10 were viewed as tendencies.

Results and Discussion

There were no differences ($P > 0.05$) in the initial body weight, final body weight, ADG, ADFI, and FCR of pigs among the dietary treatments (Table 3). Compared with the control group, no differences ($P > 0.05$) were observed in the loin-eye area and average backfat thickness, while the backfat thickness decreased 8% in pigs fed the MIXO diet compared with pigs fed the RAPO diet. Pigs fed the RAPO diet and MIXO diet tended to have an increased dressing percentage compared to pigs fed the control diet ($P = 0.069$).

Table 3 Effects of dietary supplementation with vegetable oil on the growth performance and carcass quality of growing-finishing pigs

Item[2]	Dietary treatment[1]			SEM	*P*-value
	Control	RAPO	MIXO		
Growth performance					
Initial bodyweight, kg	45.95	45.25	45.06	0.952	0.868
Final bodyweight, kg	88.18	87.83	88.65	1.173	0.565
ADG, kg/d	1.01	1.02	1.04	0.062	0.986
ADFI, kg/d	2.84	2.77	2.83	0.186	0.743
FCR	2.79	2.78	2.73	0.138	0.743
Carcass characteristics					
Dressing percentage, %	70.12	73.11	71.65	0.805	0.069
Loin-eye area, cm^2	54.38	56.05	53.52	2.663	0.796
Backfat thickness, mm	23.17	24.99	22.95	0.418	0.147

[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil
[2]ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio

The serum levels of TC and BUN were not affected by dietary treatment (P >0.05) (Table 4). The RAPO and MIXO diets decreased serum TG, GLU, HDL, and LDL levels and increased serum INS levels (P <0.05). Compared with RAPO diet, MIXO diet decreased serum Scr and LDL levels, whereas serum HDL levels increased (P <0.05).

Table 4 Effects of dietary supplementation of vegetable oils on serum parameters of growing-finishing pigs

Item[2]	Dietary treatment[1]			SEM	*P*-value
	Control	RAPO	MIXO		
TC, mmol/L	3.77	3.93	3.93	0.45	0.803
TG, mmol/L	1.51[a]	0.91[b]	0.75[c]	0.07	0.027
LDL, mmol/L	2.92[a]	1.87[b]	1.33[c]	0.05	0.009
HDL, mmol/L	1.50[a]	0.65[c]	1.14[b]	0.13	0.008
GLU, mmol/L	5.46[a]	4.47[b]	4.58[b]	0.21	0.037
Scr, μmol/L	151.67[a]	152.00[a]	108.50[b]	3.11	0.003
BUN, mmol/L	5.45	5.50	5.47	0.45	0.316
INS, mIU/L	5.12[b]	12.96[a]	10.66[a]	0.52	0.009

[a, b, c]Values with different letters within a row are significantly different (P <0.05)
[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil
[2]TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; GLU, glucose; Scr, serum creatinine; BUN, blood urea nitrogen; INS, insulin

The effects of dietary vegetable oils on meat quality are shown in Table 5. The experimental diets had no effect on the pH at 24 h, meat colour (including the L*, a* and b* values), water loss ratio, and shear force (P >0.05). Compared with the control diet, the MIXO diet decreased drip loss and cooking loss (P <0.05), while there was no difference between the MIXO and RAPO diets. Compared with the control diet, the RAPO and MIXO diets increased the IMF content by 19.25% and 20.11%, respectively (P <0.05).

Table 5 Effects of dietary supplementation with vegetable oils on the meat quality of growing-finishing pigs

Item	Dietary treatment[1]			SEM	*P*-value
	Control	RAPO	MIXO		
pH_{24}	5.91	6.00	5.98	0.18	0.935
Intramuscular fat, %	3.48[a]	4.15[b]	4.18[b]	0.38	0.042
Drip loss, %	6.30[a]	5.56[ab]	4.95[b]	0.51	0.036
Water loss, %	38.29	37.29	38.00	1.04	0.346
Cooking loss, %	23.32[a]	22.49[ab]	21.64[b]	1.51	0.047
Shear force, N	64.22	65.57	66.68	4.39	0.060
Colour parameters					
Lightness, L*	45.68	44.98	45.54	0.93	0.855
Redness, a*	6.34	6.48	6.29	0.14	0.440
Yellowness, b*	14.66	13.97	14.04	0.53	0.130

[a, b, c]Values with no common letters within a row are significantly different (P <0.05)
[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil

Compared with the control diet, both the MIXO and RAPO diets decreased the levels of C16:0, C18:0 and total saturated fatty acids (SFAs) (P <0.05), increased the total monounsaturated fatty acid (MUFA) content (P <0.05), and tended to increase the C18:1 content in the SAT of pigs (P = 0.068) (Table 6). In the LM, the levels of C16:0 and C18:0 and the total SFA content were decreased, and the levels of C12:0 and C18:1 and the total MUFA content were increased, by feeding with the MIXO and RAPO diets (P <0.05) (Table 7). Feeding with the MIXO and RAPO diets increased the linoleic acid (C18:2n-6), α-linolenic acid (C18:3n-3), total n-6 PUFA, and total n-3 PUFA levels (P <0.05) in both SAT and LM, resulting in a marked decrease in the n-6:n-3 PUFA ratio (P <0.05). The α-linolenic acid content and lower n-6:n-3 PUFA ratio in the SAT and LM of pigs fed the MIXO diet were higher than those in pigs fed the RAPO diet (P <0.05), but there was no difference in linoleic acid content in the LM (P >0.05).

Table 6 Effects of dietary supplementation of vegetable oil on the fatty acid composition in the subcutaneous adipose tissue (SAT) of growing-finishing pigs (% of total fatty acids)

Item	Dietary treatment[1]			SEM	*P*-value
	Control	RAPO	MIXO		
Saturated fatty acids, SFA					
Butyric acid, C4:0	0.22	0.18	0.20	0.04	0.313
Caprylic acid, C8:0	0.01	0.01	0.01	0.01	0.754
Capric acid, C10:0	0.06	0.05	0.04	0.01	0.068
Lauric acid, C12:0	0.07	0.06	0.06	0.01	0.558
Myristic acid, C14:0	1.12	1.02	1.02	0.04	0.560
Pentadecylic acid, C15:0	0.06	0.04	0.05	0.01	0.136
Palmitic acid, C16:0	23.1[a]	19.53[b]	19.27[b]	1.04	0.037
Margaric acid, C17:0	0.37	0.39	0.39	0.03	0.499
Stearic acid, C18:0	14.17[a]	11.94[b]	11.8[b]	0.94	0.042
Arachidic acid, C20:0	0.26[a]	0.21[b]	0.20[b]	0.02	0.037
Heneicosanoic acid, C21:0	0.02	0.02	0.02	0.01	0.745
Behenic acid, C22:0	0.04	0.02	0.03	0.01	0.426
Monounsaturated fatty acids, MUFA					
Mace acid, C14:1	0.01	0.01	0.01	0.01	0.820
Pentaenoic acid, C15:1	0.03	0.02	0.02	0.01	0.406
Palmitoleic acid, C16:1	1.64	1.49	1.61	0.11	0.115
Heptadecenoic acid, C17:1	0.29	0.28	0.33	0.04	0.760
Oleic acid, C18:1	40.02	41.41	40.91	0.64	0.068
Gadoletic acid, C20:1n-9	0.79	0.82	0.86	0.04	0.489
Nervonic acid, C24:1	0.12	0.12	0.14	0.04	0.753
Polyunsaturated fatty acids, PUFA					
Linoleic acid, C18:2n-6	15.24[a]	18.78[b]	16.67[c]	1.18	0.022
γ-linolenic acid, C18:3n-6	0.03	0.04	0.03	0.01	0.921
α-Linolenic acid, C18:3n-3	0.90[a]	2.38[b]	5.32[c]	0.24	0.048
Eicosadienoic acid, C20:2	0.62	0.63	0.75	0.03	0.075
Dihomo-γ-linolenic acid, C20:3n-6	0.08	0.08	0.09	0.01	0.531
Arachidonic acid, C20:4n-6	0.19	0.21	0.21	0.02	0.717
Dihomo-α-linolenic acid, C20:3n-3	0.29[a]	0.26[a]	0.48[b]	0.07	0.047
Eicosapentaenoic acid, C20:5n-3	0.03[a]	0.03[a]	0.06[b]	0.01	0.008
Docosahexaenoic acid, C22:6n-3	0.05	0.04	0.05	0.01	0.591
Total SFA	39.5[a]	33.47[b]	33.11[b]	1.91	0.045
Total MUFA	42.9[a]	44.13[b]	43.88[b]	0.70	0.025
Total PUFA	17.43[a]	22.45[b]	23.66[b]	1.09	0.039
n-6 PUFA	15.54[a]	19.11[b]	17.00[b]	0.74	0.045
n-3 PUFA	1.27[a]	2.71[b]	5.91[c]	0.26	0.001
n-6:n-3 PUFA[2]	12.24[a]	7.05[b]	2.88[c]	0.33	0.001

[a, b, c]Values with no common letters within a row are significantly different (P <0.05)
[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil
[2]n-6: n-3 PUFA, [sum of n-6 PUFAs/sum of n-3 PUFAs] ratio

Table 7 Effects of dietary supplementation of vegetable oils on the fatty acid composition in the longissimus dorsi muscle (LM) of growing-finishing pigs (% of total fatty acids)

Item	Dietary treatment[1]			SEM	*P*-value
	Control	RAPO	MIXO		
Saturated fatty acids, SFA					
Butyric acid, C4:0	1.24	1.18	1.29	0.13	0.456
Capric acid, C10:0	0.13	0.15	0.19	0.04	0.764
Undecanoic acid, C11:0	0.03	0.05	0.03	0.01	0.091
Lauric acid, C12:0	0.19[a]	0.28[b]	0.26[b]	0.03	0.042
Myristic acid, C14:0	1.34	1.31	1.20	0.05	0.311
Palmitic acid, C16:0	24.62[a]	23.75[b]	22.31[c]	0.47	0.041
Margaric acid, C17:0	0.19	0.19	0.20	0.02	0.564
Stearic acid, C18:0	15.30[a]	12.48[b]	12.23[b]	0.30	0.034
Arachidic acid, C20:0	0.44	0.40	0.38	0.07	0.220
Monounsaturated fatty acids, MUFA					
Pentadecenoic acid, C15:1	0.54	0.51	0.58	0.14	0.147
Palmitoleic acid, C16:1	3.04	3.03	2.92	0.22	0.445
Heptadecenoic acid, C17:1	0.19	0.18	0.23	0.03	0.429
Gadoletic acid, C20:1n-9	0.68	0.74	0.73	0.02	0.411
Oleic acid, C18:1	43.9[a]	45.67[b]	45.25[b]	1.14	0.029
Polyunsaturated fatty acids, PUFA					
Linoleic acid, C18:2n-6	6.97[a]	8.58[b]	8.26[b]	0.46	0.031
α-Linolenic acid, C18:3n-3	0.57[a]	1.10[b]	3.18[c]	0.08	0.023
Eicosadienoic acid, C20:2	0.35	0.24	0.29	0.04	0.072
Arachidonic acid, C20:4n-6	0.77	0.71	0.79	0.15	0.065
Total SFA	43.48[a]	39.79[b]	38.09[b]	0.84	0.035
Total MUFA	48.35[a]	50.13[b]	49.71[b]	1.18	0.036
Total PUFA	8.66[b]	10.63[b]	12.52[c]	0.68	0.044
n-6 PUFA	7.74[a]	9.29[b]	9.05[b]	0.48	0.037
n-3 PUFA	0.57[a]	1.10[b]	3.18[c]	0.09	0.001
n-6:n-3 PUFA[2]	13.58[a]	8.45[b]	2.85[c]	0.72	0.001

[a, b, c]Means within a row with no common superscript differ significantly (P <0.05)
[1]Control, basal diet; RAPO, 4.5% rapeseed oil; MIXO, 2.25% rapeseed oil and 2.25% linseed oil
[2]n-6: n-3 PUFA, [sum of n-6 PUFAs/sum of n-3 PUFAs] ratio

The expression of genes related to lipid metabolism was analysed (Figure 1), including lipoprotein lipase (*LPL*), glucose-responsive transcription factor (*ChREBP*), fatty acid synthase (*FAS*), fatty acid binding protein 4 (*FABP4*), hormone-sensitive lipase (*HSL*), stearoyl-CoA desaturase 1 (*SCD1*) and fatty acid elongase-5 (*Elovl5*). In SAT and LM, the expression levels of SCD1 and LPL were upregulated (P <0.05), and the expression levels of ChREBP and FAS were downregulated (P <0.05), by feeding with the MIXO and RAPO diets, compared with the control diet. The expression levels of *FABP4* and *Elovl5* were lower in the SAT of pigs fed the RAPO diet than in that of pigs fed the control or MIXO diet (P <0.01), whereas their expression levels were higher in the LM of pigs fed the RAPO or MIXO diets than in the pigs fed the control diet. The *HSL* expression in SAT was markedly increased by the MIXO diet compared with the control and RAPO diets, but its expression in the LM was decreased by the MIXO and RAPO diets, compared with the control diet (P <0.01).

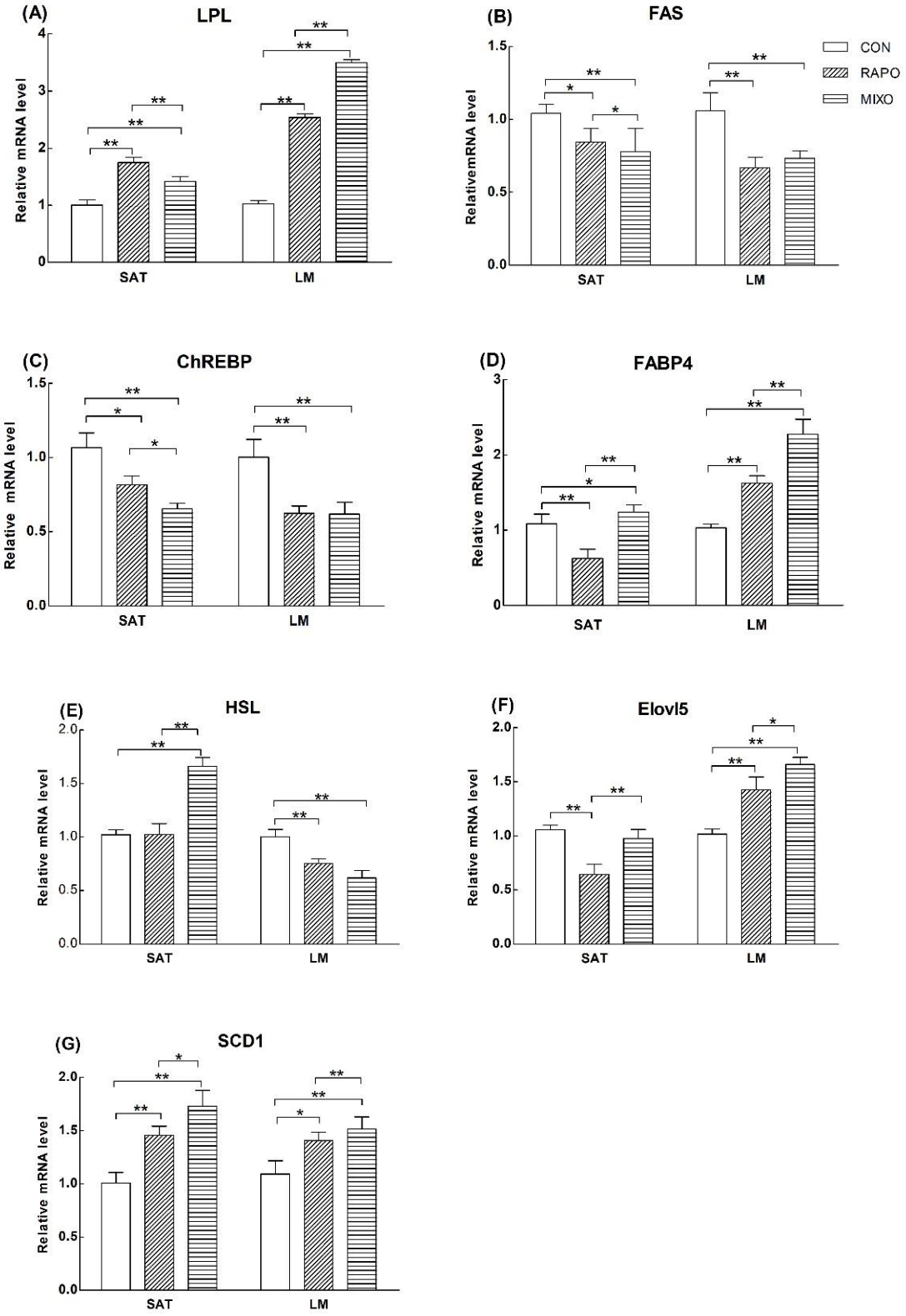

Figure 1 Relative gene expressions of (A) lipoprotein lipase (*LPL*), (B) fatty acid synthase (*FAS*), (C) carbohydrate response element-binding protein (*ChREBP*), (D) fatty acid binding protein 4 (*FABP4*), (E) hormone-sensitive lipase (*HSL*), (F) fatty acid elongase-5 (*Elovl5*), and (G) stearoyl-CoA desaturase 1 (*SCD1*) in LM (longissimus dorsi muscle) and SAT (subcutaneous adipose tissue) of pigs with dietary supplementation of 4.5% mixed oil (MIXO, n-6:n-3 PUFA ratio of 2:1) or 4.5% rapeseed oil (RAPO, n-6:n-3 PUFA ratio of 7:1). Control, basal diet. Results were presented by mean ± SD. * P <0.05; ** P <0.01

This study showed no marked effect of vegetable oil supplementation on the ADG, ADFI, FCR, loin-eye area, or backfat thickness but a tendency to increase the dressing percentage in the 42-day trial. Similar to our results, diets supplemented with 4% rapeseed oil or soybean oil (Vehovsky *et al*., 2019) or with 10% linseed oil or sunflower oil (Realini *et al*., 2010) did not affect ADG, ADFI, or carcass traits in pigs. Conversely, Nong *et al*. (2020) reported that a diet with a lower n-6:n-3 PUFA ratio (3:1 *vs*. 5:1 and 8:1) increased the F:G ratio in Heigai pigs (a local fatty breed). Duan *et al*. (2014) reported that the final body weight and ADG were substantially increased when pigs were fed a mixed oil diet with an n-6:n-3 PUFA ratio of 5:1 (0.75% linseed oil and 2.25% soybean oil). These discrepant results could be due to differences in genetic background, feeding periods, dietary composition, dietary levels of n-3 PUFAs, or environmental conditions in the previous studies. In the current study, a slight increase in the dressing percentage of the pigs was observed when the diets were supplemented with 4.5% rapeseed oil or mixed oil, which could be related to the n-3 PUFA-enriched diets improving energy utilization by increasing insulin activity (Palmquist, 2009).

The LDL is a primary cholesterol carrier in blood for delivering cholesterol to tissues and a key indicator for disease diagnosis, therapy, and monitoring of disease progression. In this study, we found that supplementation with rapeseed oil and mixed oil substantially decreased serum levels of LDL, HDL, GLU, and TG without affecting TC (especially serum LDL and TG levels, which were most significantly decreased in the mixed oil diet). This suggests that reducing the dietary n-6:n-3 PUFA ratio from 7:1 to 2:1 could further improve glucose and lipid metabolism in pigs. Consistent with a previous study *(Song et al*., 2020), a low dietary n-6:n-3 PUFA ratio effectively decreased serum concentrations of LDL and TG. Recent studies have confirmed that a low dietary ratio of n-6:n-3 PUFA and total consumption of n-3 PUFA are beneficial for improving obesity-induced inflammation and insulin resistance (Fan *et al*., 2020). In the current study, we observed that a dietary n-6:n-3 ratio of 2:1 resulted in increased expression of LPL, the rate-limiting enzyme for the clearance of circulating TGs in the LM (Wang and Eckel, 2009). However, Nguyen *et al*. (2019) reported that a decrease in the dietary n-6:n-3 PUFA ratio from 17:1 to 5:1 did not affect serum HDL, TC, or TG levels. Song *et al*. (2020) reported that dietary n-6:n-3 PUFA ratios of 2:1 and 4:1 decreased serum TC levels, while the addition of 10% sunflower oil or coconut oil tended to increase serum concentrations of HDL, LDL, and TC (Iyer *et al*., 2012). These divergent results might be partly due to differences in the total fat content and fatty acid composition of the diet.

Although the mixed oil diet reduced the serum creatinine level, the value fell in the normal physiological range (70–203 μmol/L) (Friendship *et al*., 1984), suggesting that this diet had no adverse effect. Hăbeanu *et al*. (2019) reported that diets rich in n-3 PUFAs substantially decreased the serum creatinine concentration in barrows and were related to increased retention and utilization of nitrogen. The results indicated that a dietary n-6:n-3 PUFA ratio of 2:1 could further improve the metabolism of glucose, fat, and nitrogen in finishing pigs, which would have additional potential benefits for the health and productivity of pigs.

The effect of dietary lipids on IMF content and backfat thickness in pigs is still controversial. Diets supplemented with lipids consisting of 1% linseed oil and 1–5% poultry fat (Huang *et al*., 2020), 4% rapeseed oil or soybean oil (Vehovsky *et al*., 2019), or a low dietary n-6:n-3 PUFA ratio (Nong *et al*., 2020) had no effect on the IMF content or backfat thickness of pigs. However, Li *et al*. (2015) reported that the IMF content was higher in pigs fed diets with n-6:n-3 PUFA ratios of 2.5:1, 5:1, and 10:1 than pigs fed diets with an n-6:n-3 PUFA ratio of 1:1 but had no effect on backfat thickness. In our study, the IMF content increased by 19.25% and 20.11% in the LM of pigs fed the RAPO and MIXO diets, respectively. These findings suggest that the dietary n-6:n-3 PUFA ratio could be a key factor of vegetable oils in affecting the IMF content of pork; ratios of 2:1 and 7:1 were suitable for improving the IMF content.

In the current study, *ChREBP*, a glucose-responsive transcription factor, was significantly downregulated, indicating that the *de novo* fatty acid synthesis pathway promoted by glucose could be downregulated (Gregoire *et al*., 1998). The downregulation of *FAS* expression was accompanied by the upregulation of *FABP4* in the RAPO and MIXO diets, implying that vegetable oil supplementation would increase fatty acid transport and decrease fatty acid synthesis in the LM (Boss *et al*., 2015; Wang *et al*., 2015). In addition, although the dietary vegetable oils had no significant effect on the backfat thickness of pigs, feeding with the MIXO diet decreased the backfat thickness by 8%, compared with the RAPO diet. Pigs fed the mixed oil diet showed increased expression of *HSL* in SAT, indicating an increase in the lipolysis of TGs stored in adipose tissues (Watt *et al*., 2006). This is consistent with the findings of Song et al. (2020). The diets with different n-6:n-3 PUFA ratios had different effects on fat deposition in SAT and LM, suggesting that fat deposition in these tissues might be differentially regulated by diets.

Although the underlying mechanism can be complex, several studies have reported that high n-3 PUFA levels can affect fat metabolism by regulating the expression of lipogenic genes (Ogłuszka *et al*., 2017). A diet rich in the n-3 PUFAs stimulated the expression of genes involved in the regulation of muscle metabolism and affected the interaction between lipogenesis and oxidative processes in the longissimus thoracis muscle of pigs (Vitali *et al*., 2018). N-3 PUFAs selectively drove the expansion of adipocyte numbers to control

adipogenesis by activating the n-3 fatty acid receptor, FFAR4 (Hilgendorf *et al*., 2019). The heritability estimates for linoleic and arachidonic acids in IMF and SAT reveal that the linoleic acid metabolic pathway facilitates an efficient increase in the IMF content in pigs (Gol *et al*., 2019).

In our study, the pH value, meat colour, water loss ratio, and shear force in the LM were not affected by vegetable oil supplementation in the diets. One unanticipated finding was that the drip loss and cooking loss were substantially decreased in the LM of pigs fed the mixed oil diet. Similarly, Song *et al*. (2020) reported that cooking loss in the LM was decreased by linseed-supplemented diets with 2:1 and 4:1 ratios of n-6:n-3 PUFA, while the pH, colour parameters, drip loss, and shear force were not influenced. However, studies have reported that incorporating 1% linseed oil (Huang *et al*., 2020) or 4% rapeseed oil or soybean oil (Vehovsky *et al*., 2019) to the diets had no influence on the IMF content, meat colour, shear force, or drip loss in pigs; even dietary inclusion of 20% rapeseed meal had no effect on drip loss in the loin and belly (Skugor *et al*., 2019). In the current study, the decreased drip loss and cooking loss of meat might be related to the increased IMF content, which will eventually improve the edible value of pork. Overall, it can be concluded that a 4.5% mixed oil in the diet had a positive effect on meat quality.

Previous studies have shown that linseed oil is richer in α-linolenic acids compared to other plant-derived oils, which increases the n-3 PUFA content in tissues and modifies the lipid profile (De Tonnac *et al*., 2017). We observed that an increase in the linoleic acid and α-linolenic content in the LM and SAT substantially decreased the n-6:n-3 PUFA ratio. In SAT, the rapeseed oil diet with an n-6:n-3 PUFA ratio of 7:1 increased the α-linolenic and linoleic acid levels by 164% and 23%, respectively, resulting in a low n-6:n-3 PUFA ratio (7.05 *vs*. 12.24), and the mixed oil diet with an n-6:n-3 PUFA ratio of 2:1 increased the α-linolenic and linoleic acid levels by 490% and 9%, respectively, resulting in a lower n-6:n-3 PUFA ratio (2.88 *vs.* 12.24), compared with a basal diet rich in carbohydrates. Similarly, dietary n-6:n-3 PUFA ratios of 4:1 and 2:1 led to a nearly 4-fold increase in the α-linolenic acid content in adipose tissue (Song *et al*., 2020). In LM, the rapeseed oil diet increased the α-linolenic and linoleic acid levels by 92% and 23%, respectively, and the n-6:n-3 PUFA ratio decreased from 13.58 to 8.45; the mixed oil diet increased the α-linolenic and linoleic acid levels by 457% and 18%, respectively, and the n-6:n-3 PUFA ratio decreased to 2.85. Consistent with our results, diets supplemented with rapeseed oil substantially increased the linoleic acid and α-linolenic acid proportions in IMF (Vehovsky *et al*., 2019), and more n-6 and n-3 PUFAs accumulated in the LM when pigs were fed a 10% linseed diet (Huang *et al*., 2008). These results suggest that both rapeseed and mixed oil diets can substantially increase the α-linolenic acid and linoleic acid levels and reduce the n-6:n-3 PUFA ratio in tissues. However, a mixed oil diet with an n-6:n-3 PUFA ratio of 2:1 appeared to be more effective in improving fatty acid composition, which is supported by the opinion that dietary fatty acids can be incorporated into these tissues with few modifications (Leikus *et al*., 2018).

In addition, the rapeseed and mixed oil supplementation decreased the content of C16:0, C18:0 and total SFAs and increased the total MUFA and C18:1 content in SAT and LM, and the C12:0 level in the LM. Similarly, the contents of C14:0, C16:0, C16:1, and C18:1 in the LM and SAT of pigs were affected by the vegetable oil type or n-6:n-3 PUFA ratios (Nuernberg *et al*., 2005; Realini *et al*., 2010). In the current study, we found that dietary vegetable oils increased the expression of *SCD1* in SAT and in LM, and the expression of *Elovl5* in the SAT of pigs fed the RAPO diet was lower than that in the SAT of pigs fed the MIXO diet. These two enzymes can catalyse the synthesis of MUFAs and long-chain fatty acids in tissues (Gregory *et al*., 2011). Fan *et al*. (2020) reported that reducing the n-6:n-3 PUFA ratio in the diet is a key determinant in promoting n-3 PUFA biosynthesis and subsequent lipidome modifications. Considering the effects on IMF, blood glucose and insulin, it can be reasonably postulated that the synthesis and conversion of fatty acids was also affected by the diets. This finding is supported by other studies. Doran *et al*. (2006) found that vegetable oil types differentially affected SCD activity and SCD protein expression in the muscle and adipose tissue, and SCD catalysed the formation of MUFAs (mainly 16:1 and 18:1) from the corresponding SFAs (16:0 and 18:0). The dietary n-6:n-3 PUFA ratios of 1:1-5:1 produced different effects on the expression level of genes involved in fatty acid metabolism in the LM and SAT of pigs, such as fatty acid transport protein (*FATP*)-1, *FATP*-4 and *ACC* (Li *et al*., 2015), which encode key enzymes for the *de novo* biosynthesis of fatty acids. The n-3 PUFAs resulted in increased expression of genes encoding fatty acid synthesis, desaturation, and elongation in the longissimus thoracis muscle of pigs, including *ACC*, *ChREBP*, *ELOVL6*, *FASN*, and *SCD* (Vitali *et al*., 2018). Thus, the effects of dietary PUFAs on the fatty acid profile of muscle and adipose tissue can include both direct incorporation into and effects on fatty acid metabolism, and the metabolism is different in both tissues (Hernández-Sánchez *et al*., 2013).

Moreover, we observed that although supplementation with vegetable oil substantially increased the α-linolenic acid content, it had little effect on the DHA and EPA levels. Alpha-linolenic acid is capable of undergoing a series of desaturation and elongation reactions leading to its conversion to EPA (C20:5n-3) and DHA (C22:6n-3). However, due to the limited activity of Δ6 desaturase generating stearidonic acid (SDA; C18:4n-3), a rate-limiting enzyme for α-linolenic acid substrates, the conversion to EPA and DHA is poor (Li *et al*., 2017). Dietary

SDA increased the red blood cell EPA content by approximately 17%, whereas the efficiency of α-linolenic acid was approximately 0.1% (Harris *et al*., 2008).

Conclusions

Diets supplemented with 4.5% rapeseed or mixed oil did not affect growth performance and carcass characteristics, but increased the IMF content in the LM, improved the fatty acid composition of the LM and SAT, and promoted the metabolic health of finishing pigs. However, the mixed oil diet with an n-6:n-3 PUFA ratio of 2:1 had multiple benefits for meat quality, including reduced drip loss and cooking loss, increased α-linolenic and linoleic acid content, and a decreased n-6:n-3 PUFA ratio. A mixed oil diet with an n-6:n-3 PUFA ratio of 2:1 is an effective strategy for improving meat quality and promoting the metabolic health of pigs.

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Authors' contributions

SSJ and JXL wrote this manuscript. FW, CCT, and DLYEA conducted the major experiments. GHZ, SSJ, and LF performed parts of the experiments and analyses. JXL and GHZ reviewed and edited the manuscript.

Conflict of Interest Declaration

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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