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Effects of linseed oil supplementation duration on fatty acid profile and fatty acid metabolism-related genes in the muscles of Chinese crested white ducks

Yang Zhang,*,[1] Zhi Cao,*,[1] Laidi Wang,* Bingqiang Dong,* Shangzong Qi,* Xinlei Xu,* Qiang Bao,* Yu Zhang,* Qi Xu,* Guobin Chang,* and Guohong Chen*,†,[2]

*Key Laboratory of Animal Genetics and Breeding and Molecular Design of Jiangsu Province, College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu Province 225009, China; and †Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, Jiangsu Province, 225009, China

ABSTRACT Meat rich in polyunsaturated fatty acids is considered beneficial to health. Supplementing the diet with linseed oil promotes the deposition of polyunsaturated fatty acids (**PUFAs**) in poultry, a conclusion that has been confirmed multiple times in chicken meat. However, fewer studies have focused on the effects of dietary fatty acids on duck meat. Therefore, this study aims to evaluate the effects of the feeding time of a linseed oil diet on duck meat performance and gene expression, including meat quality performance, plasma biochemical indicators, fatty acid profile, and gene expression. For this study, we selected 168 Chinese crested ducks at 28 days old and divided them into three groups, with 56 birds in each group. The linseed oil content in the different treatment groups was as follows: the control group (0% flaxseed oil), the 14d group (2% linseed oil), and the 28d group (2% linseed oil). Ducks in the two experimental groups were fed a linseed oil diet for 28 and 14 days at 28 and 42 days of age, respectively. The results showed that linseed oil had no negative effect on duck performance (slaughter rate, breast muscle weight, and leg muscle weight) or meat quality performance (pH, meat color, drip loss, and shear force) ($P > 0.05$). The addition of linseed oil in the diet increased plasma total cholesterol and high-density lipoprotein cholesterol levels ($P < 0.05$), while decreasing triglyceride content ($P < 0.05$). Furthermore, the supplementation of linseed oil for four weeks affected the composition of muscle fatty acids. Specifically, levels of α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid were increased ($P < 0.05$), while eicosatetraenoic acid content was negatively correlated with flaxseed oil intake ($P < 0.05$). qRT-PCR analysis further revealed that the expression of *FATP1*, *FABP5*, and *ELOVL5* genes in the breast muscle, as well as *FABP3* and *FADS2* genes in the thigh muscle, increased after four weeks of linseed oil supplementation ($P < 0.05$). However, after two weeks of feeding, *CPT1A* gene expression inhibited fatty acid deposition, suggesting an increase in fatty acid oxidation ($P < 0.05$). Overall, the four-week feeding time may be a key factor in promoting the deposition of n-3 PUFAs in duck meat. However, the limitation of this study is that it remains unknown whether longer supplementation time will continue to affect the deposition of n-3 PUFAs. Further experiments are needed to explain how prolonged feeding of linseed oil will affect the meat quality traits and fatty acid profile of duck meat.

Key words: linseed oil, duck, polyunsaturated fatty acid, fatty acid transport protein genes, fatty acid binding protein gene

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INTRODUCTION

In China, duck meat is just after pork and chicken meat in terms of popularity. However, under conditions of similar nutritional quality, duck meat has a higher polyunsaturated fatty acids (**PUFAs**) content than other meats and, thus, serves as a potential source of high-quality protein and PUFAs for humans (Zhao et al., 2018). PUFAs, as bioactive compounds, are divided into 2 families, n-3 PUFAs and n-6 PUFAs. Linoleic acid (**LA**) and α-linolenic acid (**ALA**) are precursor substances for the synthesis of n-6 PUFAs and n-3 PUFAs, respectively (Kouba and Mourot, 2011). In vertebrates, they are essential, and can only be consumed through food. And n-3 PUFAs have been found

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[1]These authors contributed equally to this article.

[2]Corresponding author: ghchen2019@yzu.edu.cn

to help inhibit inflammation and lipid production (Djuricic and Calder, 2021). The n-6: n-3 PUFAs ratio associated with the greatest health benefit ranges from 5 to 10 (Simopoulos, 2002; Zárate et al., 2017), yet this ratio in duck meat exceeds 15 (Wang et al., 2022). Excessive supplementation with n-6 PUFAs increases the risk of inflammation, obesity, and cancer (Marventano et al., 2015; Naughton et al., 2016). Therefore, to increase the n-3 PUFA content in duck meat, we can change the structure of the PUFAs in their diet, which will reduce the ratio of n-6: n-3. Although fish oil is the best source of n-3 PUFAs, its high cost and negative effects on the sensory quality of chicken meat make its use more cautious (Lopez-Ferrer et al., 1999). However, linseed sidesteps these problems. In particular, linseed oil, which is rich in n-3 PUFAs, has replaced fish oil as the new dietary oil supplement for poultry (Kumar et al., 2019).

Studies on linseed oil or linseed have impacted poultry in a number of aspects, including production performance, meat quality, serum biochemical indicators, and muscle fatty acid (**FA**) composition (Liu et al., 2011; Kalakuntla et al., 2017; Wang et al., 2022). The addition of linseed or linseed oil to poultry diets can promote the enrichment of n-3 PUFAs in muscle and eggs, reduce the n-6: n-3 ratio, and provide consumers with meat rich in n-3 PUFAs (Kalakuntla et al., 2017; Li et al., 2017; Shahid et al., 2019; Ngo Njembe et al., 2021). The abundance of ALA in linseed reduces blood triglyceride levels, which is important for maintaining the health of poultry (Liu et al., 2011; Li et al., 2017; Shahid et al., 2019). After absorption in the intestine, the PUFAs contained in linseed oil are transported through the circulatory system to various tissues. The uptake of essential FA by various tissues of organism is largely dependent on protein-mediated channels in the cell membrane (Canbay et al., 2007). Fatty acid transport protein (**FATP**), solute carrier 27A (**SLC27A**), cluster of differentiation 36 (**CD36**), and fatty acid binding protein (**FABP**) play a dominant role in the overall transmembrane transport process (Canbay et al., 2007). In addition, ducks have a strong FA conversion ability in the liver (Gregory and James, 2014) and can convert ALA in food to DHA through fatty acid desaturase (**FADS**) and fatty acid elongation enzyme (**ELOVL**), and then supply it to other tissues through blood circulation (Koletzko et al., 2019; Nie et al., 2021).

Previous scholars have comprehensively explored the effects of linseed oil on fat deposition within the liver of Chinese crested white ducks through transcriptomic, proteomic, and metabolomic analyses, ultimately revealing key genes that regulate the synthesis and metabolic pathways of hepatic n-3PUFAs (Wang et al., 2022; Zhang et al., 2022). However, these findings do not explain the metabolic process of flaxseed oil in the muscles of Chinese crested white ducks. Therefore, to elucidate the possible regulatory effects of exogenous PUFAs on the muscle lipids of Chinese crested white ducks, herein, we assessed the effects of linseed oil feeding time (2 or 4 weeks) on production performance, plasma biochemical indices and muscle FA composition in these ducks. Furthermore, we analyzed the changes in FA metabolism-related gene (transport, synthesis, and oxidation) mRNA expression in the breast and thigh muscles. Collectively, the primary aim of this study is to provide a reference for the metabolism of muscle n-3 PUFAs from the perspective of gene expression and diet.

MATERIAL AND METHODS

Experimental Design and Management

All experimental procedures performed in this study were approved by the Institutional Animal Committee of Yangzhou University (Permit Number: YZUDWSY, Government of Jiangsu Province, China). This experiment was carried out in strict accordance with a completely randomized block experimental design, and the initial body weight of the duck was regarded as a block factor. China Zhenjiang Tiancheng Agricultural Science and Technology Co., Ltd. provided the Chinese crested white ducks used for the experiment. Xilinguolemeng Hongjing Yuan Oil and Fat Company Limited produces linseed oil by physically pressing flaxseeds, resulting in a high-quality oil. A total of 168 healthy Chinese crested white ducks (28 days old, mean weight 753.35 ± 12.94 g) were selected and randomly divided into 3 groups with 4 replicates in each group and 14 ducks (half male and half female) in each replicate. The composition of the duck diets was established based on the nutritional needs of meat ducks, and diets comprised a corn-soybean meal-type diet. The ducks in the control group were fed a basal diet (2% duck oil) between 28 and 56 d of age. Duck fat in the diet of the experimental group was replaced by 2% linseed oil. Ducks in the experimental group were fed diets containing linseed oil from 28 d and 42 d of age for 28 d and 14 d, respectively. The Nutritional levels of substances such as Ca, CP and Total phosphorus in the diet were detected according to the AOAC standard method (AOAC, 2005). The crude fat content in the feed was expressed in ether extract and determined by the Soxhlet extraction method. The specific diet and nutrient compositions are shown in Table 1.

Gas chromatography was used to determine the FA compositions of the feed and raw oils (Table 2). The ALA and n-3 PUFAs contents of the linseed oil were significantly higher than those in the duck oil. Therefore, the linseed oil was able to provide diets rich in n-3 PUFAs. During the experimental period, all ducks were raised in pens in the house with approximately 6 cm high mats comprising sawdust and rice hulls on the floor. Each pen was duplicated using an automatic water-feeding device and feeder to meet the conditions of free drinking and feeding. The average temperature in the house during the experimental period was 28°C and the humidity was 67%. All ducks were weighed at the beginning and end of the experiment; the amount of feed

Table 1. Composition and main characteristics of feed.

Ingredient %	Control group	Linseed group
Corn	47.16	47.16
Soybean meal	24.23	24.23
Wheat flour	17.66	17.66
Rice bran	5.00	5.00
Duck fat	2.00	0.00
Linseed oil	0.00	2.00
Dicalcium phosphate	1.65	1.65
Limestone	1.00	1.00
Salt	0.30	0.30
Premix[1]	1.00	1.00
Total	100	100
Nutrient level[2]		
AME (MJ/kg)	11.31	11.31
CP	17.52	17.52
EE	5.76	5.76
CF	3.59	3.59
Ca	0.89	0.89
Total phosphorus	0.75	0.75
Lysine	0.90	0.90

Abbreviations: AME, apparent metabolizable energy; CF, crude fiber; CP, crude protein; EE, ether extract.

[1]Vitamin and mineral premix supplied kilogram diet: vitamin A, 9,000 IU; vitamin D_3, 3,000 IU; vitamin E, 79 mg; vitamin B_2, 8 mg; vitamin K_3, 2 mg; pantothenic acid, 3.2 mg; niacin, 11 mg; folic acid, 1.5 mg; biotin,1 mg; Co, 1 mg; Mn, 49 mg; Cu, 6 mg; Zn, 60 mg; I, 2 mg; Se, 0.18 mg.

[2]Calculated values.

consumed and the number of ducks that died during the experiment were recorded for each group.

Sampling

At the end of the study period, 6 ducks (half male and half female) were randomly selected from each replicate. After 12 h of fasting, the ducks were weighed (**BW**) and the average daily gain (**ADG**) was calculated, as were feed consumption and the feed conversion ratio (**FCR**). Venous blood was collected from the left wing of the duck and stored in sodium heparin anticoagulant tubes. The separated plasma (3,000 rpm centrifuged for 15 min) was stored at −20°C for subsequent analysis.

The ducks were electrically stunned and killed by exsanguination. The feathers were removed to determine the slaughter weight. Subsequently, the animals were dissected and the tissues and organs were separated to calculate all eviscerated and half-eviscerated weights. Slaughter performance was conducted based on Chinese poultry production performance terminology and determination methods (China National Standard, 2004). Four samples were collected from each right breast (*pectoralis major*) and thigh (*biceps fermoris*) muscle and stored at −80°C for subsequent analysis. Finally, the left breast (*pectoralis major*) muscles and left thigh (*biceps fermoris*) muscles were separated as a whole, weighed, and stored at 4°C for 24 h to be used for subsequent analysis of meat quality and nutritional indicators.

Detection of Meat Quality

A portable pH meter (DELTA320, Mettler Toledo, Zurich, Switzerland) was used to determine the pH of the muscles. In brief, the pH meter glass electrode was inserted ∼1 cm into the breast and thigh muscles. Then, the upper left corner, middle, and lower right corner of each muscle sites were tested. The flesh color of the breast and thigh muscles was measured sites using a colorimeter (CR-400, Konica Minolta, Japan), including its brightness, redness, and yellowness, scored using the CIELAB system.

Next, breast and thigh muscles were cut into small 30 g squares and the water-holding capacity of the muscles was measured for 5 min using a pressure device (YYW-2, Ruifeng, Beijing, China); the drip loss rate was calculated. We divided the remaining muscles into 2 portions of similar size. One portion was cooked in a water bath (20 min at 80°C) and used for shear force determination after the muscles were cooled. A muscle tenderometer (C-LM3B, Tenovo, Beijing, China) was employed to measure the shear force of the muscle. The sample was first cut into strips (3 cm long × 1 cm

Table 2. Fatty acid composition of duck diets.

Composition of fatty acid	Content (g/100 g total fatty acids)			
	Control group	Linseed group	Duck oil	Linseed oil
SFA	30.95	26.51	28.34	10.52
MUFA	38.23	31.46	46.50	20.28
PUFA	30.82	42.03	25.16	69.20
LA(C18:2n-6)	28.63	27.53	23.33	16.50
GLA(C18:3n-6)	ND	ND	0.06	0.61
DGLA(C20:3n-6)	0.12	0.07	0.11	0.07
ARA(C20:4n-6)	0.18	0.12	0.34	0.07
Total n-6 PUFA	28.93	27.72	23.84	17.24
ALA(C18:3n-3)	1.53	14.00	0.95	51.68
EPA(C20:3n-3)	ND	ND	0.01	0.05
DHA(C22:6n-3)	0.13	0.12	0.14	0.13
Total n-3 PUFA	1.67	14.12	1.17	51.92
n-6/n-3	17.36	1.97	20.40	0.33
Total fatty acids (g/100g)	5.00	4.72	70.32	68.13

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; MUFA, monounsaturated fatty acid; ND means not detected; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Note: The unit of total fatty acids is g/100 g fresh sample.

Table 3. Sequences of primer pairs of mRNA.

Gene	Forward (5′−3′)	Reverse (5′−3′)	Product (bp)
ELOVL5	ATGAACTGGGTGCCTTGTGG	CAGATACGGACGCATTGCTG	117
FADS2	CTGTTGAGTATGGCAAGAAGAA	GGAAATACACGGGGATGAG	102
CPT1A	GCCATCTTGCTCTACCG	TAAACATCCGCTCCCAC	106
CPT1B	GCTACGGCGTCTCCTACATC	GATGTTCCTCCCGAAACGCT	107
FABP5	GATGGCAGAAAAACTCAGACGC	TCCATCCACAAGTTTCCGTGT	111
FATP1	GCCGGATTTTGCCCAATGTT	CGCAGGGGATCCTGTTGATT	154
ACSL1	GGCTCTGCGTTACTCCAC	GCATAGCATCCCTGTTCG	107
FABP3	ATGGTGGAGGCTTTCGTG	GATGGTGGTGGGTTTGGT	126
ACTB	TATGTCGCCCTGGATTTCG	ACAGGACTCCATACCCAAGAA	165

Abbreviations: ACSL1, acyl-CoA synthetase long-chain family member 1; ACSL4, acyl-CoA synthetase long-chain family member 4; ACTB, beta-actin; CPT1A, carnitine palmitoyltransferase 1A; CPT1B, carnitine palmitoyltransferase 1 B; ELOVL5, elovl fatty acid elongase 5; FABP3, fatty acid binding protein 3; FABP5, fatty acid binding protein 5; FADS2, fatty acid desaturase 2; FASN, fatty acid synthase; FATP1, fatty acid transport protein 1; PPARα, peroxisome proliferator-activated receptor alpha.

wide × 1 cm high) and then sheared perpendicular to the direction of the fibers to calculate the shear force of the muscle (200 mm/min, 15 kg). Finally, connective tissues were removed from the remaining samples and the meat was crushed using a meat mincer (Teste, Nanjing, China). The water, intermuscular fat, protein, and collagen contents were measured using a FoodScan meat analyzer (Foss, Hilleroed, Denmark) (Anderson, 2007).

Plasma Biochemical Indicators

The concentrations of aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), **AST/ALT,** total cholesterol (**TC**), triglyceride (**TG**), high-density lipoprotein cholesterol (**HDL-C**), and low-density lipoprotein cholesterol (**LDL-C**) in the plasma samples were measured using a Roche-Hitachi Cobas 8000 (Roche, Basel, Switzerland) and an accompanying assay kit (Roche, Basel, Switzerland). The assay was performed according to the manufacturer's instructions.

Fatty Acid Analysis

Approximately 100 mg of the breast and thigh muscles were sheared for hydrolysis; saponification and methyl esterification were performed according to a previously described protocol (Christie, 1993). After the sample was stratified, the supernatant was separated and filtered through a 0.4-μm membrane. Subsequently, the samples were assessed with a Trace1310-ISQ gas chromatograph-mass spectrometer (Thermo, Waltham, MA); an equal volume of specimens (1 μL) was injected into an HP-88 column (100 m × 0,25 mm × 0.2 μm, Agilent, California) for FA separation. The operating procedure comprised an initial temperature of 100°C (15 min), which was increased to 190°C (25 min) at a rate of 15°C/min, and then to 235°C (4 min) at a rate of 2.5°C/min. The detector and injector temperatures were 280°C and 240°C, respectively. The carrier gas was helium and the flow rate of the carrier gas was maintained at 1.0 mL/min. Finally, the results were compared with the retention times and peak areas of standards (Sigma, California) to detect long-chain FA in the samples.

RNA Extraction and RT-qPCR Verification

Total RNA was extracted from breast and thigh muscle samples using TRIzol reagent (Invitrogen, Carlsbad, CA), followed by reverse transcription of cDNA according to the procedure of the FastKing gDNA Dispelling RT SuperMix kit (Beijing Tiangen Biochemical Technology Co., Ltd., China). The sequences of fatty acid elongase enzyme 5 (***ELOVL5***), fatty acid desaturase 2 (***FADS2***), fatty acid transport protein 1 (***FATP1***), acyl-coenzyme A synthetase 1 (***ACSL1***), carnitine palmitoyltransferase 1A (***CPT1A***), carnitine palmitoyltransferase 1 B (***CPT1B***), fatty acid binding protein 3 (***FABP3***), fatty acid binding protein 5 (***FABP5***), and beta-actin (***ACTB***) were obtained from the NCBI website, and primers were designed using Primer Premier 5.0 software (Table 3).

Primer sequences were synthesized by DynaBio (Nanjing) Technology Co., Ltd (China). The target gene was amplified by PCR using cDNA as a template and the primers were tested for availability by agarose gel electrophoresis. The QuantStudio 5 Real-Time Fluorescent Quantitative PCR Detection System (Applied Biosystems, Foster, CA) and ChamQTM SYBR qPCR Master Mix reagents were used to detect mRNA expression of genes within the samples. The 10 μL reaction system comprised 5 μL of SYBR Premix Ex TaqTM II (2×), 0.4 μL of upstream and downstream primers (10 μmol/L), 0.4 μL of ROX Reference Dye (50 ×), 2 μL of cDNA as template, and 2.2 μL of ddH_2O. The reaction procedure for all genes was predenaturation at 95°C for 30 s, followed by 95°C for 10 s and 60°C for 30 s, for a total of 40 cycles. *ACTB* was used as an internal reference gene and each assay was repeated in triplicate. The relative mRNA expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method, and the results were normalized for comparison.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 26.0) to assess the chi-square test. One-way repeated measures analysis of variance (**ANOVA**) was performed to test the significance of differences after the requirements were satisfied, and the least-significant difference (**LSD**) method was used for post hoc multiple

Table 4. Effect of dietary linseed oil supplementation for 14 or 28 d on the growth performance of ducks slaughtered at 56 d of age.

Items[1]	Control	14 d	28 d	SD	*P* value
BW (kg)	1.45	1.36	1.38	0.07	0.147
FI (kg)	2.67	2.62	2.70	0.12	0.688
ADG (g/d)	69.35	60.63	63.31	6.34	0.190
FCR	3.87	4.35	4.26	0.34	0.106
Breast meat (%)	8.68	8.82	8.86	1.15	0.131
Thigh meat (%)	8.30[a]	8.07[a]	8.63[b]	0.64	0.013

Abbreviations: ADG, average daily gain; BW, body weight; FCR, feed conversion rate; FI, feed intake.

The results are expressed as mean ± standard deviation ($n = 4$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

[1]Breast meat %, Relative weight of the breast muscles on each side (weight after peeling /live weight × 100); Thigh meat %, Relative weight of the thigh muscles on each side (weight after peeling /live weight × 100).

Table 6. Effect of dietary linseed oil supplementation for 14 or 28 d on breast meat quality of ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
L*	40.57	39.66	45.31	5.16	0.291
a*	16.87	16.39	16.60	1.18	0.874
b*	6.02	6.03	6.54	1.41	0.865
pH	5.99	6.02	6.10	0.09	0.233
Drip loss (%)	24.24	21.86	25.52	7.33	0.869
Shear force (N)	27.37	29.64	25.30	7.34	0.776
Protein (%)	23.35	23.52	23.74	0.36	0.135
Intramuscular fat (%)	1.84	2.02	1.83	0.21	0.297
Collagen (%)	0.78	1.28	1.22	0.52	0.109
Moisture (%)	75.48	74.18	75.15	0.93	0.275

Abbreviations: a*, redness value; b*, yellowness value; L*, lightness value.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

comparisons. A $P < 0.05$ was considered statistically significant.

RESULTS

Growth and Slaughter Performance

The variations in body weight, FI, ADG, FCR, and breast muscle weight of ducks fed the basal diet or supplemented with linseed oil were negligible ($P > 0.05$; Table 4). However, 4-wk supplementation of linseed oil increased the weight of the duck thigh muscles ($P < 0.05$). Furthermore, the slaughter performance of the ducks was not affected by linseed oil ($P > 0.05$; Table 5).

Meat Quality and Nutritional Indicators of Breast and Thigh Muscles

No correlation was observed between the duck meat quality and linseed oil content. Meat quality characteristics such as meat color, pH, and tenderness of breast and thigh muscles did not change with increasing duration of linseed oil feeding ($P > 0.05$; Tables 6 and 7). Although a 4-wk feeding period increased the water content in the thigh muscle of ducks ($P < 0.05$), other nutritional indicators of meat quality, such as proteins, fat and collagen, were not affected ($P > 0.05$).

Table 7. Effect of dietary linseed oil supplementation for 14 or 28 d on thigh meat quality of ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
L*	39.45	38.87	38.14	3.43	0.887
a*	14.34	13.34	14.93	2.27	0.684
b*	5.47	6.67	7.28	1.69	0.459
pH	6.82	6.95	6.89	0.16	0.556
Drip loss (%)	19.73	22.07	21.63	7.33	0.909
Shear force (N)	31.99	28.94	38.12	10.16	0.477
Protein (%)	21.90	21.83	21.82	0.36	0.907
Intramuscular fat (%)	3.30	3.16	3.32	0.52	0.866
Collagen (%)	0.78	0.74	0.67	0.12	0.398
Moisture (%)	74.84[ab]	74.56[a]	75.56[b]	0.71	0.049

Abbreviations: a*, redness value; b*, yellowness value; L*, lightness value.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

Table 5. Effect of dietary linseed oil supplementation for 14 or 28 d on carcass composition and meat yield of ducks slaughtered at 56 d of age.

Items[1]	Control	14 d	28 d	SD	*P* value
All eviscerated weight (g)	734.02	742.62	729.17	59.49	0.908
Dressing percentage (%)	85.54	85.23	85.18	1.42	0.871
Half eviscerated percentage (%)	60.83	61.25	61.94	2.15	0.637
All eviscerated percentage (%)	53.74	54.50	55.33	2.41	0.479
Breast muscle percentage (%)	15.04	16.71	16.38	2.12	0.262
Thigh muscle percentage (%)	16.73	16.49	16.58	1.80	0.968
Abdominal fat percentage (%)	1.66	1.22	1.86	0.93	0.469

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

[1]Dressing percentage (%) = carcass weight (g) / live weight (g) × 100%; All eviscerated percentage (%) = All eviscerated weight (g) / live weight (g) × 100%; Half eviscerated percentage (%) = Half eviscerated weight (g) / live weight (g) × 100%; Breast muscle percentage (%) = breast muscle weight (g) / all eviscerated weight (g) × 100%; thigh muscle percentage (%) = thigh muscle weight (g) / all eviscerated weight (g) × 100%.

Table 8. Effect of dietary linseed oil supplementation for 14 or 28 d on plasma biochemical indicators of ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
ALT(U/L)	28.25	25.08	29.67	8.37	0.401
AST(U/L)	38.00	29.33	35.17	9.59	0.090
AST/ALT	1.43	1.19	1.19	0.26	0.138
TC (mmol/L)	4.02^{a}	5.00^{b}	4.62^{b}	0.65	0.000
TG (mmol/L)	0.80^{a}	0.67^{b}	0.65^{b}	0.16	0.040
HDL-C(mmol/L)	2.31^{a}	2.67^{b}	2.64^{b}	0.35	0.001
LDL-C(mmol/L)	1.10	1.24	1.21	0.16	0.059

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

Plasma Biochemical Indicators

The plasma TG, TC, and HDL-C levels in ducks treated with linseed oils differed from those treated with the control ($P < 0.05$; Table 8). However, no differences were observed between the different treatment groups. In contrast, the level of plasma TG decreased with the prolongation of feeding time with linseed oil ($P < 0.05$; Table 8).

Fatty Acid Composition of Breast and Thigh Muscle

Compared to the control group, supplementation with linseed oil for 2 or 4 wk increased the amount of n-3 PUFAs in the breast muscle by 0.43 mg and 1.39 mg, respectively, and DHA content by 0.22 mg and 0.72 mg, respectively ($P < 0.05$; Table 9). The ALA and EPA contents increased progressively with feeding time, with significantly increased levels observed after 4 wk of supplementation ($P < 0.05$); however, the 2-wk treatment time did not promote their deposition within the breast muscle ($P > 0.05$). Moreover, differences were not observed in the total FA, total saturated FA, total mono-unsaturated FA, total polyunsaturated FA, ETA, ARA, LA, and n-6 PUFAs levels between the different treatment groups ($P > 0.05$). Furthermore, the consumption of exogenous FA linseed oil affected the n-6/n-3 ratio of the duck breast muscle, exhibiting a linear reduction with increasing feeding time ($P < 0.05$).

Dietary supplementation with linseed oil also impacted the n-3 PUFAs, LA, EPA, and ETA content in duck thigh muscle ($P < 0.05$; Table 10). That is, a positive linear relationship was observed in the abundance of n-3 PUFAs, ALA, and EPA with feeding time ($P < 0.05$). In contrast, dietary linseed oil reduced the abundance of ETA in thigh muscles ($P < 0.05$). No differences were observed in total FA, total saturated FA, total monounsaturated FA, total polyunsaturated FA, DHA, ARA, LA, or n-6PUFAs contents were observed among the groups ($P > 0.05$). However, the n-6/n-3 ratio decreased linearly within the thigh muscle with increased linseed oil feeding time ($P < 0.05$). Overall, the 4-wk linseed oil treatment had a greater beneficial effect on breast and thigh muscle FA concentrations than 2-wk treatment.

Table 9. Effect of dietary linseed oil supplementation for 14 or 28 d on fatty acid composition of breast muscle from ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
TFA (mg/g)	50.72	42.78	54.05	8.80	0.135
Total SFA	23.45	19.85	24.97	3.63	0.106
Total MUFA	8.46	6.79	7.28	1.45	0.292
Total PUFA	18.81	16.14	21.8	3.81	0.126
n-3	1.12^{a}	1.55^{b}	2.51^{b}	0.43	0.000
ALA	0.29^{a}	0.43^{a}	0.65^{b}	0.17	0.015
EPA	0.21^{a}	0.29^{a}	0.54^{b}	0.12	0.003
DHA	0.61^{a}	0.83^{b}	1.33^{c}	0.25	0.000
n-6	17.26	14.24	18.84	3.27	0.168
LA	8.42	6.84	10.16	2.16	0.129
ETA	0.41	0.37	0.49	0.06	0.067
ARA	8.44	7.04	8.19	1.28	0.229
n-6/n-3	15.35^{a}	9.23^{b}	7.50^{b}	3.23	0.000

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; LA, linoleic acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid; SFA, saturated fatty acid; TFA, total fatty acid.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 24$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

Table 10. Effect of dietary linseed oil supplementation for 14 or 28 d on fatty acid composition of thigh muscles from ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
TFA (mg/g)	112.15	62.53	91.65	40.61	0.156
Total SFA	44.09	24.76	33.89	14.93	0.129
Total MUFA	30.70	14.08	25.18	15.34	0.228
Total PUFA	37.35	23.69	32.58	10.91	0.125
n-3	2.37^{a}	2.40^{a}	4.47^{b}	1.27	0.007
ALA	0.73^{a}	0.84^{a}	2.67^{b}	1.07	0.002
EPA	0.22^{a}	0.22^{a}	0.28^{b}	0.04	0.050
DHA	1.42	1.34	1.53	0.36	0.724
n-6	34.40	20.92	27.68	10.17	0.112
LA	19.76	10.43	16.51	7.98	0.175
ETA	0.39^{a}	0.29^{b}	0.32^{b}	0.06	0.041
ARA	14.24	10.19	10.86	2.46	0.059
n-6/n-3	14.26^{a}	8.73^{b}	6.34^{b}	3.63	0.000

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic acid; LA, linoleic acid; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid; SFA, saturated fatty acid; TFA, total fatty acid.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 24$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

Changes in Fatty Acid-Related Gene Expression in Breast and Thigh Muscle

The expression of genes related to FA transport and synthesis, such as *FABP5, FATP1,* and *ELOVL5,* was

Table 11. Effect of dietary linseed oil supplementation for 14 or 28 d on the expression of genes related to fatty acid metabolism in breast muscle of ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
ELOVL5	1.00[a]	1.27[a]	1.78[b]	0.34	0.009
FADS2	1.00	0.90	0.72	0.30	0.135
CPT1A	1.00	0.98	0.85	0.36	0.697
CPT1B	1.00	0.76	0.85	0.25	0.201
FABP5	1.00[a]	1.07[a]	2.06[b]	0.48	0.001
FATP1	1.00[a]	1.24[a]	1.66[b]	0.31	0.002
ACSL1	1.00	0.83	0.73	0.39	0.247
FABP3	1.00	1.35	1.14	0.28	0.164

Abbreviations: ACSL1, acyl-CoA synthetase long-chain family member 1; CPT1A, carnitine palmitoyltransferase 1A; CPT1B, carnitine palmitoyltransferase 1 B; ELOVL5, elovl fatty acid elongase 5; FABP3, fatty acid-binding protein 3; FABP5, fatty acid-binding protein 5; FADS2, fatty acid desaturase 2; FATP1, fatty acid transport protein 1.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

increased in the breast muscle of ducks fed dietary linseed oil for 4 wk ($P < 0.05$; Table 11). However, the expression of *CPT1A, CPT1B, FADS2, FABP3*, and *ACSL1* was not affected ($P > 0.05$).

Four weeks of treatment with linseed oil also promoted the expression of *FADS2* and *FABP3* in the thigh muscles ($P < 0.05$; Table 12). On the other hand, the expression of *CTP1A* mRNA was only affected following 2 wk of feeding ($P < 0.05$). Meanwhile, no differences were observed in the expression of *ACSL1, FABP5, CPT1B*, and *ELOVL5* mRNA among the groups ($P > 0.05$). After scrutinizing the aforementioned results, we show it in the form of a model diagram in order to understand more intuitively the changes in fatty acid levels in muscle tissue and the regulation of genes associated with fatty acid metabolism, consequent to the addition of linseed oil (Figure 1).

Table 12. Effect of dietary linseed oil supplementation for 14 or 28 d on the expression of genes related to fatty acid metabolism in thigh muscle of ducks slaughtered at 56 d of age.

Items	Control	14 d	28 d	SD	*P* value
ELOVL5	1.00	0.86	0.85	0.31	0.655
FADS2	1.00[a]	1.18[a]	1.54[b]	0.29	0.042
CPT1A	1.00[a]	2.00[b]	1.35[ab]	0.46	0.000
CPT1B	1.00	1.16	0.79	0.24	0.057
FABP5	1.00	1.01	0.99	0.17	0.949
FATP1	1.00	0.96	1.17	0.21	0.149
ACSL1	1.00	1.09	1.16	0.19	0.367
FABP3	1.00[a]	1.03[a]	2.06[b]	0.32	0.000

Abbreviations: ACSL1, acyl-CoA synthetase long-chain family member 1; CPT1A, carnitine palmitoyltransferase 1A; CPT1B, carnitine palmitoyltransferase 1 B; ELOVL5, elovl fatty acid elongase 5; FABP3, fatty acid-binding protein 3; FABP5, fatty acid-binding protein 5; FADS2, fatty acid desaturase 2; FATP1, fatty acid transport protein 1.

Control, no linseed oil added; 14 d, addition of 2% linseed oil for 2 wk; 28 d, addition of 2% linseed oil for 4 wk.

The results are expressed as mean ± standard deviation ($n = 12$), and the means within the same row with different superscript letters are significantly different ($P < 0.05$).

DISCUSSION

Performance and Meat Quality

In this study, supplementation of the diets of Chinese crested white ducks with linseed oil did not negatively affect growth performance nor slaughter performance, and induced changes in BW, FI, FDG, and FCR that were similar to those reported in other similar studies (Kanakri et al., 2017; Li et al., 2017). In addition, research has found that continuous feeding of linseed increases the weight of Beijing duck breast muscles (Shahid et al., 2019). Although our results showed that feeding linseed oil for 4 wk increased the weight of the Chinese crested white duck thigh muscles, there was no significant change in breast muscle weight. This difference may be related to the type of duck, feeding duration, and source of dietary fatty acids. In addition, dietary supplementation with linseed oil did not affect the pH of Chinese crested white duck breast or thigh muscles. The meat color, drip loss, tenderness, and nutritional indicators were not affected by the duration of linseed oil feeding. These results agree with those of previous studies (Kartikasari et al., 2012; Shahid et al., 2019).

Plasma Biochemical Indicators

AST and ALT are 2 important transaminases located in the cytoplasm and mitochondria of hepatocytes, and their activity in plasma is often used to assess the state of the liver. That is, if the metabolic homeostasis of hepatocytes is disrupted, the cell membrane permeability increases, leading to passage of transaminases from the liver into the blood through capillaries, and this change is directly reflected by the AST/ALT ratio (Hall and Cash, 2012). We found that linseed oil did not affect the liver function of Chinese crested white ducks, and the plasma AST/ALT ratio tended to decrease with increasing feeding time. Levels of n-3 polyunsaturated fatty acids are significantly associated with liver health (Jin et al., 2019; Zhang et al., 2022), and n-3 PUFAs promote hepatic FA transport (Wang et al., 2022) and inhibit fat deposition within hepatocytes, thus contributing to the maintenance of liver health.

Plasma HDL-C levels reflect the metabolic state of cholesterol (Gracia-Rubio et al., 2021). More specifically, reverse cholesterol transport is thought to be a major function of HDL-C. Cholesterol in peripheral cells becomes bound by HDL-C and transported directly through the bloodstream to the liver, where extrahepatic sources of HDL-C are specifically taken up by hepatocyte-expressed SR-B1 receptors into hepatocytes for metabolism and, ultimately, converted into bile acids for excretion (Shen et al., 2018; Yu et al., 2019). In our study, the concentrations of HDL-C and TC in the plasma of Chinese crested white ducks increased linearly with the duration of linseed oil feeding. TC represents the sum of all cholesterol contained in plasma lipoproteins; thus, its increase provides the basis for higher

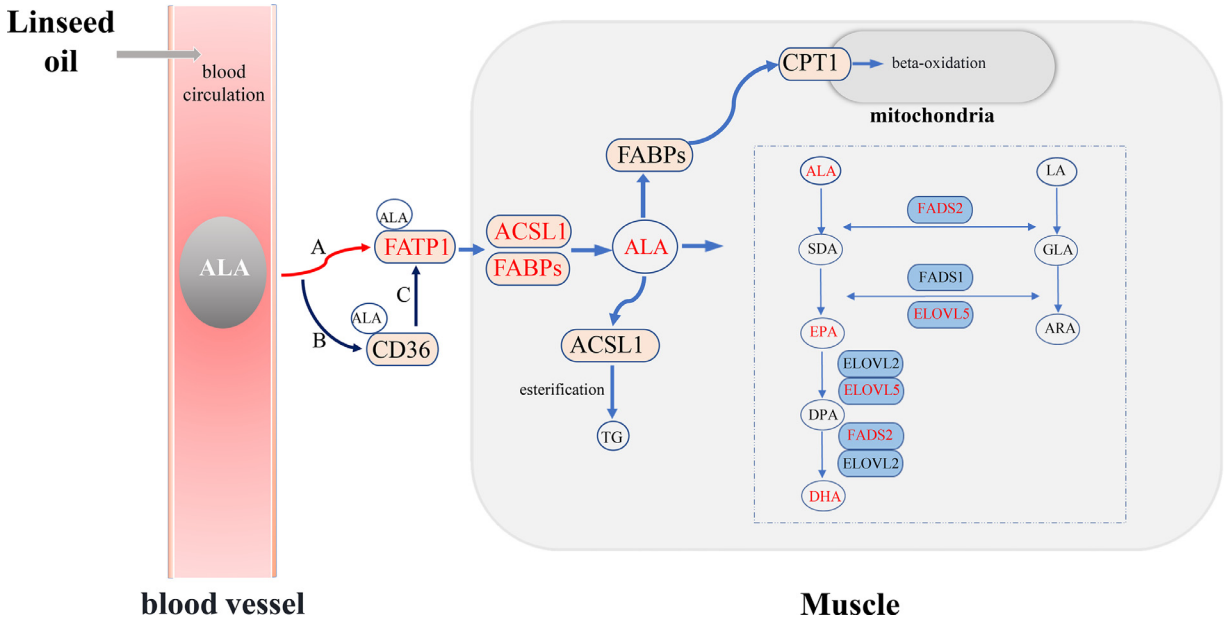

Figure 1. Effects of linseed oil on polyunsaturated fatty acids and genes related to fatty acid metabolism in duck muscle tissue. Blue boxes indicate genes, orange boxes show the genes and the proteins encoded by these genes, transparent boxes indicate metabolites, and red fonts indicate upregulation. Note: A, ALA binds directly to the FATP1 protein and enters the cell under the co-action of the FABPs family and ACSL1; B, ALA first binds to the CD36 protein and is then transmitted to FATP1 to enter cells; C, the ALA bound to the CD36 protein is delivered to FATP1.

cholesterol clearance in peripheral cells. As such, TC and TG levels reflect changes in blood lipids. Dietary PUFA inhibit de novo liver lipogenesis and by consequence the triglyceride synthesis and export in blood (Ayerza and Coates, 2007; Shahid et al., 2019). This is similar to our finding that the content of TG decreases with increasing levels of PUFAs.is similar to our findings. n-3 PUFAs, especially DHA, have a positive effect on the improvement of HDL-C levels (Chan et al., 2006), which inhibits the activation of cholesterol regulatory element binding proteins, thereby regulating the transcription of genes related to FA metabolism in various tissues, leading to a decreased plasma TG concentration (Worgall et al., 1998; Torrejon et al., 2007). In addition, plasma TG levels affect the stability of HDL-C, thus, low concentrations of TG are more favorable for the operation of serum HDL-C (Worgall et al., 1998; Chan et al., 2006). These results strongly support our finding that linseed oil can reduce cholesterol levels in peripheral cells and maintain liver health in ducks.

Absorption of Dietary Fatty Acids

Different structures of dietary FA have been reported as an effective means to modify the FA profile of duck meat (Li et al., 2017; Shahid et al., 2019; Wang et al., 2022; Zhang et al., 2022). Exogenous PUFAs absorbed from the small intestine are esterified to form TGs, which enter the lymphatic system with lipoproteins to form celiac particles that eventually enter the bloodstream only to be transported to various tissues, where they affect the FA composition (Rymer and Givens, 2005). In addition, we found that the total FA level in the pectoral muscles of ducks was lower than that in the thigh muscles, which has also been reported in broiler chickens (Betti et al., 2009). This phenomenon is intricately linked to the types of muscle fibers present and the distribution of fatty acids across various muscle tissues (Kriketos et al., 1995; Gonzalez-Esquerra and Leeson, 2001; Sanosaka et al., 2008).

Changes in Fatty Acid Profile and Fatty Acid-Related Genes in Breast Muscle

The main function of *FABP* family members is to specifically bind FA. Subsequently, FA are transported from the cell membrane to sites of TG and phospholipid synthesis or catabolism (Guaita-Esteruelas et al., 2018). ALA from linseed oil can either enter the cell directly via *FATP1*, or it can first bind to the CD36 molecule on the cell membrane, from which it is delivered to *FATP1* and transported into the cell (Stahl et al., 2001; Richards et al., 2006). ALA-entering cells may be catalyzed by *ACSL1* to undergo esterification, or is transported to organelle membranes via the *FABP* family (Guaita-Esteruelas et al., 2018). Meanwhile, dietary linseed oil contributes to the expression of *CD36* in breast muscle (Wang et al., 2022). Indeed, we found that feeding linseed oil for 4 wk promoted the transcription of *FATP1* and *FABP5* within the breast muscle of ducks, leading to accelerated ALA, EPA, and DHA deposition, which is consistent with previous reports (Li et al., 2017; Wang et al., 2022).

Downstream EPA and DHA are derived from the conversion of ALA, a process that heavily relies on the family of fatty acid desaturases and elongases (Cormier et al., 2014). Dietary linseed oil is known to contribute to increased expression levels of *FADS1, FADS2, ELOVL2,* and *ELOVL5* within the chicken breast (Gou et al., 2020). We also observed upregulated expression of

ELOVL5 in the duck breast muscle, thus, providing an essential enzyme for EPA and DHA production within this tissue. Surprisingly, previous studies have found a lack of correlation between *FADS1* and *FADS2* activity and the formation of long-chain PUFAs (Haug et al., 2014), which might be due to differences in the species and FA concentrations.

Changes in Fatty Acid Profile and Fatty Acid-Related Genes in Thigh Muscle

The *CPT1* gene is thought to be a key regulator of fatty acid β-oxidation (Van Weeghel et al., 2018); that is, lipid coenzyme A, formed by *ACSL1* esterification, can enter the inner mitochondrial membrane, catalyzed by *CPT1*, for subsequent β-oxidation (Skiba-Cassy et al., 2007; Morash et al., 2009). Our study found that linseed oil feeding for 2 wk had no effect on the n-3 PUFAs content of duck thigh muscles, and changes in FA transporter protein genes stabilized without significant increases, which is in line with the current study findings for breast muscle. However, *CPT1A* gene activity significantly increased after 2 wk of n-3 PUFAs supplementation. Previous studies have suggested that PUFAs may be responsible for the increased *CPT1A* activity (Morash et al., 2009). Rather than reducing fat deposition by inhibiting FA biosynthesis, linseed oil promotes β-oxidation of FA (Ibrahim et al., 2018), resulting in slightly lower total FA content within the thigh muscles following 2 wk of supplementation, compared with the control group.

It is generally accepted that the conversion of n-3 and n-6 PUFAs is accomplished by the same FA desaturase and elongase, and that there is competition between the 2 families of PUFAs, with an increase in one inevitably leading to a decrease in the other (Kartikasari et al., 2012). However, *FADS2* has a higher affinity for n-3 PUFAs (Poureslami et al., 2010), and n-3 enriched flaxseed oil stimulates its expression. In our study, *FABP3* and *FADS2* mRNA expression was upregulated after 4 wk of linseed oil feeding, as was the abundance of ALA and EPA in duck thigh muscle. The enrichment of EPA would have saturated the FA desaturase or lengthening enzyme that produces ETA, resulting in decreased ETA content, similar to previous studies (Kanakri et al., 2017; Kumar et al., 2019). However, feeding time did not impact DHA concentrations in the thigh muscle, as was also found in studies with Cherry Valley ducks and Shaoxing ducks, which may be related to enzyme competition or oxidative metabolism of DHA (Liu et al., 2011; Li et al., 2017).

In conclusion, our research has shown that the n-3 PUFAs contained within flaxseed oil can reduce cholesterol levels within the extrahepatic tissues of Chinese Crested White ducks and maintain a healthy liver. Moreover, a strong correlation was observed between dietary FAs and the FA profiles within the breast and thigh muscles of ducks. Thus, replacing duck fat with linseed oil rich in n-3 PUFAs for 4 wk promoted the deposition of n-3 PUFAs within the muscle of ducks. This process is influenced by a combination of genes, exogenous FAs, and lipid metabolism pathways; however, the concentration of FAs predominates. The accumulation of ALA in ducks promotes the activity of FA transport and transcription of synthesis genes, thereby regulating the deposition of n-3 PUFAs in muscles. Therefore, supplementation of linseed oil for 2 wk did not provide ducks with sufficient levels of ALA to influence the FA profile of the muscle, a finding that emphasizes the dependence of the n-3 PUFAs content in duck meat on ALA concentrations. Although exogenous FAs are the main regulators of the muscle n-3 PUFA content, their influence on the liver cannot be ignored. This requires more understanding of the potential regulatory relationship between liver and muscle fatty acid metabolomics.

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DISCLOSURES

The authors declare no conflicts of interest.

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