



Effects of dietary supplementation with oleic acid on growth performance, dietary fat utilization, serum and intestinal lipid metabolic parameters, and enterocyte lipid droplet metabolism in Pekin ducks

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ABSTRACT

This study aimed to investigate the effects of a diet supplemented with different levels of oleic acid (OA) on growth performance, serum biochemical parameters, nutrient utilization, and intestinal lipid metabolism in Pekin ducks. A total of 350 fourteen-d-old male ducks were randomly assigned to the following five iso-nitrogenous and heteroenergetic dietary treatment groups: 0.00% (**control**), 0.25%, 0.50%, 0.75%, and 1.00% OA groups. The experiment lasted 28 days. The findings indicated that neither growth performance nor nutrient utilization was affected by OA supplementation ($P > 0.05$). The 0.50% OA group displayed the lowest serum triglyceride (TG) levels among all treatment groups, with significantly lower values compared to both the 0.25% and 0.75% OA groups ($P < 0.05$). Moreover, the activities of lipid droplet (LD)-degrading enzymes in the jejunal mucosa, such as adipose triglyceride lipase (ATGL), showed a significant inverse linear relationship ($P < 0.05$); carboxylesterase 2 (CES2) activity exhibited a proportional dose-dependent increase ($P < 0.05$); and lysosomal acid lipase (LAL) activity was negatively correlated with the increased concentration of OA in the diet ($P < 0.05$). Moreover, the mRNA expression levels of the LD formation-related genes *PLIN2* were significantly higher in the 0.50% OA group compared to the 0.25% and 0.75% OA groups ($P < 0.05$). The mRNA expression of LD degradation-related genes, the *PNPLA2* expression in the 0.25%, 0.50%, and 0.75% OA groups and *LPL* expression in all OA groups were downregulated ($P < 0.05$) when compared with those in the control group. These results suggested that dietary supplementation with OA, especially at a level of 0.50%, may decrease the serum TG content and promote lipid deposition in the jejunum in Pekin ducks by regulating the formation and degradation of enterocyte LDs.

Introduction

China accounts for over 70% of global duck production and consumption, with Pekin ducks being a major component (Liu et al., 2017). Pekin ducks, known for roughage tolerance, can be provided with many low-cost and unconventional feed components in compound feed, enabling reduced competition with other poultry for grains (Liu et al., 2024). As Pekin ducks exhibit rapid growth, particularly during the developmental phase, their energy requirements are substantial (Baiao and Lara, 2005). To ensure an adequate energy supply when incorporating low-energy unconventional feed ingredients, a significant amount of oil or fat must be included in compound feed (Ravindran et al., 2016;

Alhotan, 2021; Lian et al., 2024). However, diets enriched with excessive oil may not be fully digested and absorbed due to the limited secretion of lipase and bile acids (BAs) in ducks, potentially resulting in dietary lipid wastage (de Melo et al., 2017; Jiao et al., 2018; Zeng et al., 2023; Wei et al., 2024). Consequently, to address this challenge, effective lipid nutrition regulation strategies are required to maximize fat utilization efficiency and support the health of ducks.

A diet containing high levels of unsaturated fatty acids (UFAs) can enhance lipid digestion and absorption within the digestive system, thereby improving fatty acid (FA) digestibility and increasing dietary metabolizable energy concentration (Ravindran et al., 2016; Rodriguez-Sanchez et al., 2019). Oleic acid (OA), a type of UFA abundantly

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present in olive oil, canola oil, sunflower oil, and soybean oil (Shin et al., 2012; Orsavova et al., 2015), has been reported to significantly improve lipid health in animal-derived products (Toomer et al., 2020; Vlaicu et al., 2021). Recent research indicates that *cis*-OA, a specific isomer of OA, plays a role in enhancing FAs (Prom and Lock, 2021). However, its potential to improve dietary fat utilization efficiency in Pekin ducks has not yet been investigated.

More importantly, OA undergoes diacylglycerol acyltransferase-catalyzed esterification to glycerol, which contributes to lipid droplets (LD) accumulation (Nakajima et al., 2019). LDs are highly dynamic organelles that can serve as triacylglycerol (TAG) deposits, preventing excessive lipid accumulation in cellular structures and exerting protective effects against lipotoxicity (Benador et al., 2018; Olzmann and Carvalho, 2019). Increased energy demands promote the release of FAs from LDs to provide energy (Danielli et al., 2023). Lee et al. (2002) reported that the formation of enterocyte cytoplasmic lipid droplets (cLDs) was regulated by dietary nutrient composition. A correlation between dietary supplementation with OA and enterocyte cLD metabolism in Pekin ducks has not been established. Therefore, this study was conducted to investigate the effects of dietary supplementation with different levels of OA on growth performance, dietary nutrient utilization, serum and intestinal lipid metabolic parameters, and the formation and degradation of enterocyte cLDs in Pekin ducks.

Materials and methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University, China (ethics code permit no. SAUPNZ-22-08).

Birds, diet, and management

This study randomly divided 350 fourteen-d-old male Pekin ducks with an average body weight (BW) of 604.43 ± 6.39 g into the following five groups: 0.00% (control group), 0.25%, 0.50%, 0.75%, and 1.00% OA groups. The experiment lasted 28 days, from 14 to 42d of age for ducks. Each group comprised 7 replicate cages, each housing 10 ducks. The main source of oil in the diet was 9% palm oil (purchased from Yihai Kerry Arawana Holdings Co., Ltd.). OA, purchased from Shanghai BioEngineering Co., Ltd., was supplemented as a weight percentage on the basis of the total weight of the corresponding experimental diets. Five isonitrogenous and heteroenergetic diets were formulated to exceed the nutrient requirements of Pekin ducks according to the NRC (1994) and China Agricultural Industry Standards (2012) (Table 1). The dry matter (DM), crude protein, and ether extract (EE) contents were assessed following the guidelines established by AOAC (2006), and the FA compositions of the five experimental diets were determined by gas chromatography (Table 1). All diets were provided as mash. Ducks were reared in cages (1 × 0.8 × 0.6 m) in a temperature- and humidity-controlled room. During the entire experiment, the ducks had free access to feed and water.

Sample collection and measurement

At 42d of age, the ducks were weighed after an 8-hour fasting. The BW and feed consumption were recorded and the average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated. Seven birds with an average weight of each cage were selected from each treatment. Blood samples were obtained via the jugular vein and centrifuged at 3,000 g for 15 min at 4°C, and the serum was collected and stored at -80°C until analysis of the biochemical parameters. All birds were euthanized by cervical dislocation, and jejunal mucosa samples were immediately collected and stored in liquid nitrogen for analysis of intestinal lipid metabolism.

Table 1

Composition and nutrient levels of the experimental diet (as air dry basis)

Ingredient, %	Supplemented levels of dietary oleic acid, %				
	0	0.25	0.50	0.75	1.00
Corn	42.10	42.10	42.10	42.10	42.10
Soybean meal	26.87	26.87	26.87	26.87	26.87
Flour	10.00	10.00	10.00	10.00	10.00
Wheat bran	6.58	6.58	6.58	6.58	6.58
Palm oil	9.00	9.00	9.00	9.00	9.00
Dicalcium phosphate	1.65	1.65	1.65	1.65	1.65
Limestone	1.05	1.05	1.05	1.05	1.05
Sodium chloride	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.19	0.19	0.19	0.19	0.19
L- Threonine	0.07	0.07	0.07	0.07	0.07
L- Lysine hydrochloride	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.15	0.15	0.15	0.15	0.15
Mineral premix ¹	0.50	0.50	0.50	0.50	0.50
Vitamin premix ²	0.03	0.03	0.03	0.03	0.03
Rice husk	1.41	1.41	1.41	1.41	1.41
Nutrient levels ³ , %					
ME, MJ/kg	13.44	13.43	13.61	13.91	13.99
Crude protein ³	17.73	17.75	17.75	17.76	17.76
Crude fat ³	12.26	12.49	12.89	13.22	13.30
Crude fibre	3.18	3.18	3.18	3.18	3.18
Calcium	0.85	0.85	0.85	0.85	0.85
Non-phytate phosphorus	0.40	0.40	0.40	0.40	0.40
Lysine	0.85	0.85	0.85	0.85	0.85
Threonine	0.60	0.60	0.60	0.60	0.60
Methionine	0.40	0.40	0.40	0.40	0.40
Fatty acid profiles (g/100 g of total fatty acid) on an analyzed basis ³					
Palmitic acid (C16:0)	34.23	33.76	33.40	32.72	32.14
Stearic acid (C18:0)	3.80	3.80	3.73	3.68	3.63
Oleic acid (C18:1n-9)	38.37	38.97	39.97	40.54	41.30
Linoleic acid (C18:2n-6)	20.46	20.14	19.44	19.41	19.05
Saturated fatty acid	39.77	39.31	38.89	38.10	37.55
Monounsaturated fatty acid	38.65	39.46	40.68	41.45	42.40
Polyunsaturated fatty acid	21.58	21.24	20.43	20.46	20.06
U:S ratio ⁴	1.51	1.54	1.57	1.62	1.66

¹ The trace mineral premix provided the following (per kg of diet): Fe (FeS₂·O₄·H₂O), 80 mg; Cu (CuSO₄·5H₂O), 10 mg; Mn (MnO₄·H₂O), 100 mg; Zn (ZnSO₄·H₂O), 60 mg; I (KI) 0.45 mg; Se (Na₂SeO₃), 0.3 mg.

² The vitamin premix provided the following (per kg of diet): vitamin A 8,000 IU; vitamin D₃, 2,000 IU; vitamin E, 20 IU; vitamin K₃, 1.0 mg; vitamin B₁, 0.6 mg; vitamin B₂, 8.0 mg; vitamin B₆, 3.5 mg; vitamin B₁₂, 0.01 mg; niacin, 10.0 mg; niacin, 35.0 mg; folic acid, 0.55 mg; biotin 0.18 mg.

³ The levels of crude protein and crude fat as well as the fatty acid profiles were measured values.

⁴ U:S ratio = Unsaturated fatty acid:Saturated fatty acid ratio.

Serum biochemical parameters analyses

The serum levels of the following lipid metabolism-related parameters were analyzed using an automatic biochemistry analyzer (SELECTA XL; Vital Scientific, Newton, MA, USA): glucose (Glu, F006-1-1), triglyceride (TG, A110-1-1), total cholesterol (TC, A111-1-1), total bile acid (TBA, E003-2-1), high-density lipoprotein-C (HDL-C, A112-1-1), low-density lipoprotein-C (LDL-C, A113-1-1), very-low-density lipoprotein-C (VLDL-C) and free fatty acids (FFAs, A042-2-1). Moreover, the serum leptin content (LEP, H174-1-2) was determined spectrophotometrically using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. (Nanjing Jiancheng Bioengineering Institute, China).

Dietary fat utilization

Titanium dioxide (5 g/kg) was added to the five diets as an indigestible marker. On d 44, sixteen birds from each group were selected based on average BW for a 3-day metabolic trial and then divided into 8 replicates, with 2 birds per replicate. The birds in each group were fed their corresponding experimental diets. Fresh excreta (free from contaminants, e.g., feathers and feed residues) were collected on plastic

sheets (placed under each cage) every day. The droppings from the same cage were pooled (replicate), lyophilized, ground to pass through a 0.45 mm sieve, and stored at -20°C. The DM content was determined by drying at 105 °C to constant weight (AOAC, 2006). EE was determined after extraction with 99.5% diethyl ether using an accelerated extractor (AOAC, 2006). Apparent utilization coefficients of dietary DM and EE were calculated as reported previously (Naderinejad et al., 2016; Moradi et al., 2024).

Analysis of enzyme activity related to LD degradation in the jejunal mucosa

The activities of the LD-degrading enzymes monoacylglycerol lipase (MGL, 20230901M2), hormone-sensitive lipase (HSL, 20230901H4), adipose triglyceride lipase (ATGL, 20230901A1), lysosomal acid lipase (LAL, 20230901L2), carboxyesterase 2 (CES2, 20230901C3), and lipase (LPS, 20230901B3) were determined spectrophotometrically with commercial ELISA kits (Jiangsu Meimian Industrial Co., Ltd, China). The levels of TG (A110-1-1) and FFAs (A042-2-1) in the jejunal mucosa were analyzed using an automatic biochemistry analyzer (SELECTA XL; Vital Scientific, Newton, MA, USA).

Quantification of mRNA Using qRT-PCR

Total RNA was isolated from the jejunal mucosa by using TRIzol reagent (TaKaRa, Dalian, China). The RNA concentrations were quantified using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific Inc.). After the RNA concentration was determined, 1 µg of total RNA was immediately reverse-transcribed into cDNA using the Prime Script™ RT Reagent Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. The primers for FA transport-related genes (CD36, FABP1, and FAS), LD formation-related genes (PLIN2, PLIN3, DGAT2, and ACSL1), LD degradation-related genes (PNPLA2 and LPL), and GAPDH are listed in Table 2. qRT-PCR analysis was performed using the SYBR Premix Ex Taq (Takara) with an ABI 7500 Real-Time PCR detection system (Applied Biosystems, Thermo Fisher Scientific Inc. Foster City, USA). The PCR protocol included initial denaturation at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 5 s and

Table 2
The primers for quantitative real-time PCR

Gene	Primer sequence (5'-3')	Accession No.
CD36	F: GCTGCTCGTTTGAACCTGA	XM_038183702.1
	R: TGTTCGACGATGGATGAC	
FAS	F:CAATGGATCCTCAGCTTCGC	XM_027471234.2
	R:AGCTGTTCTGGATCTTGCT	
FABP1	F: CCAGAAGGGCAAGGACATGAAGAG	XM_005023289.5
	R: TGGAGCCAGTGGTGACGGTAAC	
PLIN2	F: CGGAGTTGTGGGCAAGACTA	NM_001310418.1
	R: ACCACACGACTTCCCAAGAC	
PLIN3	F: ACTTCGTTCTGCTGGGTTC	XM_013107029.3
	R: TCTATCTGCTCTGCCTGGGA	
ACSL1	F: GCAGAACTAAGAAGCGGTAT	XM_038178440.1
	R: TAGA ACTGACAGCCAAGAGC	
DGAT2	F: CTTGTTCTCTGTCTACTCCTT	XM_005016871.5
	R: GCCAATGTGCTTCTGAAAC	
PNPLA2	F: TTTGCAGCACCTTCATCCCT	XM_038179084.1
	R: TCGGGGCAAGTTGTCTGAAA	
LPL	F:GCCTTGCAGATGTGGATCAG	XM_027446391.2
	R:AAGTTGTTGCAGCGGTCTT	
GAPDH	F:GCAGATGCTGGTGCTGAATA	XM_038180584.1
	R:CGGAGATGATGACACGCTTA	

Abbreviations: CD36, cluster of differentiation 36; FAS, fatty acid synthase; FABP1, fatty acidbinding protein 1; PLIN2, perilipin 2; PLIN3, perilipin 3; ACSL1, acyl-CoA synthetase long-chain family member 1; DGAT2, diacylglycerol o-acyltransferase 2; PNPLA2, patatin like phospholipase domain containing 2; LPL, lipoprotein lipase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

annealing at 60°C for 30 s. The conditions of the melting curve analysis were as follows: one cycle of denaturation at 95°C for 10 s, followed by an increase in temperature from 65°C to 95°C at a rate of 0.5°C/s. The data are presented in arbitrary units as relative mRNA levels. The expression levels of the target genes were normalized to those of GAPDH. The relative gene expression levels were calculated using the 2^{-ΔΔCt} method (Livak and Schmittgen, 2001).

Statistical analysis

All the statistical analyses utilized the GLM procedure of SAS 9.2 software (SAS Institute, Cary, NC, USA). Differences between groups were compared using one-way analysis of variance, followed by Tukey's post hoc test. The correlations between dietary OA levels and the indices were determined using linear and quadratic regression analyses. The results were deemed statistically significant if the *P*-value was less than 0.05.

Results

Effects of OA on growth performance and dietary fat utilization

As indicated in Table 3, there were no significant differences in the final BW, ADFI, ADG, or F/G among the five groups (*P* > 0.05). As shown in Fig. 1, all OA-supplemented groups demonstrated improved utilization of dietary fat when compared to the control group, with the 0.50% OA group showing a 3.16% elevation, although the differences were not statistically significant (*P* > 0.05). There was no notable difference in dietary DM availability among all the experimental groups (*P* > 0.05).

Effects of OA on serum lipid metabolic parameters

The effects of different dietary OA concentrations on the serum biochemical parameters of ducks were presented in Table 4. Serum TG levels were significantly lower in the 0.50% OA group than in the 0.25% and 0.75% OA groups (*P* < 0.05). The serum TBA levels in the 0.50% OA and 1.00% OA groups were lower than those in the control group (*P* < 0.05). Furthermore, the serum LEP levels in the 0.50% and 1.00% OA groups were elevated relative to those in the control group (*P* < 0.05). Linear and quadratic analyses revealed a significant negative correlation between serum TBA levels and OA supplementation (*P* < 0.05), contrasting with the positive correlation observed for LEP levels (*P* < 0.05). No significant differences were observed in the serum Glu, TC, FFA, HDL-C, LDL-C, or VLDL-C levels among the five treatment groups (*P* > 0.05).

Effects of OA on lipid metabolic parameters and LD metabolism in the jejunal mucosa

Lipid metabolism and related enzyme activities of the jejunal mucosa in ducks were detailed in Table 5. Dietary supplementation with OA did not significantly affect the FFA or TG levels in the jejunal mucosa (*P* > 0.05). However, in contrast to those in the control group, the activities of ATGL, MGL, LAL, and CES2 were markedly decreased in the OA groups (*P* < 0.05). Additionally, the activity of HSL in all OA groups, except the 0.50% OA group, was noted to be significantly lower compared to that seen in the control group (*P* < 0.05). The LPS activity in the 0.75% OA group was significantly higher than that in the control group (*P* < 0.05). Furthermore, jejunal mucosal ATGL activity in Pekin ducks showed a significant inverse linear relationship with OA supplementation level (*P* < 0.05). Conversely, CES2 activity exhibited a proportional dose-dependent increase (*P* < 0.05). Linear and quadratic regression analyses revealed that the activity of LAL was negatively correlated with the concentration of OA (*P* < 0.05).

As shown in Figs 2, 3, and 4, dietary OA supplementation did not significantly affect the mRNA expression levels of FA transport-related

Table 3
The effects of diet supplemented with oleic acid on growth performance in Pekin ducks¹

Item	Supplemented levels of dietary oleic acid, %					P-value			
	0.00	0.25	0.50	0.75	1.00	SEM	ANOVA	Linear	Quadratic
Initial weight,g/bird	605.2	604.7	604.8	604.1	603.4	2.56	0.988	0.576	0.850
Final weight,g/bird	3052	3087	3083	2993	3060	55.89	0.765	0.658	0.903
Average daily gain(ADG),g/ bird /d	84.36	85.61	85.47	82.37	84.72	1.96	0.778	0.681	0.915
Average daily feed intake(ADFI),g/ bird /d	193.4	198.3	196.9	189.2	198.6	3.68	0.350	0.941	0.975
Feed-to-gain ratio (F/G)	2.30	2.33	2.31	2.30	2.35	0.05	0.937	0.607	0.843

¹ Each value represents the mean value of 7 replicates/treatment (n = 7).
^{a,b}Mean values with unlike letters were significantly different among diets with graded OA levels (1-way ANOVA, $P < 0.05$, Tukey's post hoc test,and the linear and quadratic regression analyses were performed on the relationship between dietary OA levels and the indexes).

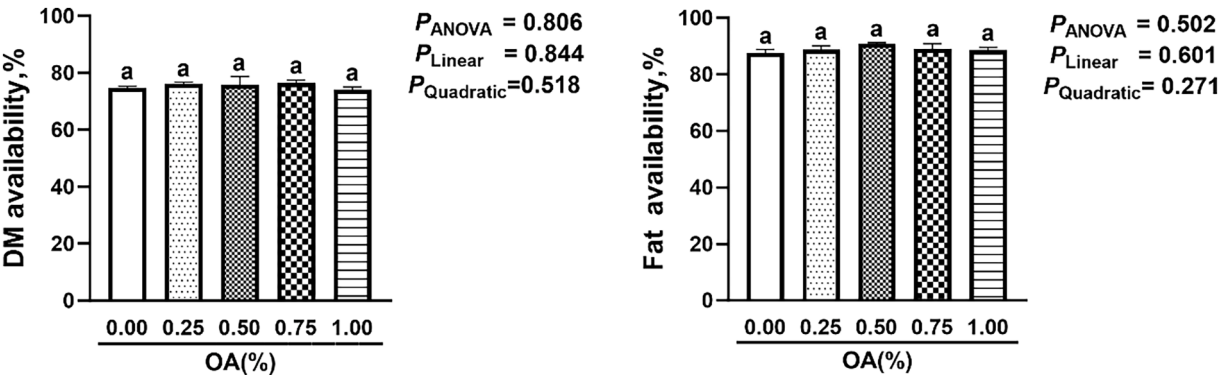


Fig. 1. The effects of diet supplemented with oleic acid on dietary nutrient utilization in Pekin ducks. Con,the basal diet; OA, the Con plus different levels of OA (0.25, c0.50, 0.75 and 1.00%). Data are means $\pm$ SEM represented by vertical bars or plot individual values(n=7).

Table 4
The effects of diet supplemented with oleic acid on serum lipid metabolic parameters in Pekin ducks¹

Items	Supplemented levels of dietary oleic acid, %					P-value			
	0.00	0.25	0.50	0.75	1.00	SEM	ANOVA	Linear	Quadratic
Glucose(Glu),mmol/L	7.51	7.87	6.71	8.39	7.34	0.39	0.057	0.897	0.992
Triglyceride(TG),mmol/L	0.62 ^{abc}	0.84 ^a	0.43 ^c	0.72 ^{ab}	0.52 ^{bc}	0.08	0.005	0.260	0.491
Total cholesterol(TC),mmol/L	3.19	2.99	3.65	3.41	3.53	0.18	0.111	0.077	0.201
Total bile acids(TBA), μ mol/L	35.28 ^a	34.01 ^a	21.28 ^b	31.44 ^a	17.91 ^b	3.23	0.001	0.002	0.010
Free fatty acids(FFA),mmol/L	0.41	0.42	0.50	0.45	0.46	0.04	0.645	0.399	0.268
High-density lipoprotein-C(HDL-c),mmol/L	1.55	1.35	1.64	1.43	1.68	0.10	0.160	0.355	0.406
Low-density lipoprotein-C(LDL-c),mmol/L	1.38	1.30	1.54	1.45	1.24	0.12	0.419	0.711	0.408
Very-low-density lipoprotein-C (VLDL-C),mmol/L	0.64	0.60	0.73	0.68	0.71	0.04	0.111	0.077	0.201
Leptin(LEP),ng/ml	1.86 ^a	2.05 ^{ab}	2.25 ^b	2.17 ^{ab}	2.39 ^b	0.12	0.044	0.003	0.013

¹ Each value represents the mean value of 7 replicates/treatment (n = 7).
^{a-c} Mean values with unlike letters were significantly different among diets with graded OA levels (1-way ANOVA, $P < 0.05$, Tukey's post hoc test,and the linear and quadratic regression analyses were performed on the relationship between dietary OA levels and the indexes).

Table 5
The effects of diet supplemented with oleic acid on the lipid metabolism of jejunal mucosa in Pekin ducks ¹

Items	Supplemented levels of dietary oleic acid, %					P-value			
	0.00	0.25	0.5	0.75	1.00	SEM	ANOVA	Linear	Quadratic
Monoacylglycerol lipase(MGL),U/L	198.2 ^a	103.9 ^d	171.2 ^b	150.0 ^c	134.1 ^c	7.13	<0.0001	0.063	0.073
Hormone sensitive lipase(HSL),U/L	693.3 ^a	363.3 ^d	651.2 ^a	542.7 ^b	465.0 ^c	24.75	<0.0001	0.090	0.207
Adipose triglyceride lipase(ATGL),U/L	183.9 ^a	85.42 ^d	162.8 ^b	131.6 ^c	113.3 ^c	6.69	<0.0001	0.040	0.079
Lysosomal acid lipase(LAL),U/L	84.74 ^a	47.22 ^d	76.02 ^b	63.28 ^c	49.48 ^d	2.25	<0.0001	0.003	0.011
Carboxylesterase 2(CES2),U/L	816.7 ^a	466.0 ^d	698.0 ^b	664.0 ^b	574.1 ^c	22.54	<0.0001	0.068	0.071
Lipase(LPS),U/g	8.44 ^b	12.1 ^b	8.02 ^b	23.59 ^a	15.72 ^{ab}	3.56	0.025	0.038	0.116
Free fatty acids(FFA), μ mol/g	4.45	3.57	4.12	6.82	4.84	0.92	0.149	0.190	0.430
Triglyceride(TG),mmol/g	0.050	0.050	0.040	0.063	0.050	0.01	0.059	0.445	0.729

¹ Each value represents the mean value of 7 replicates/treatment (n = 7).
^{a-d} Mean values with unlike letters were significantly different among diets with graded OA levels (1-way ANOVA, $P < 0.05$, Tukey's post hoc test,and the linear and quadratic regression analyses were performed on the relationship between dietary OA levels and the indexes).

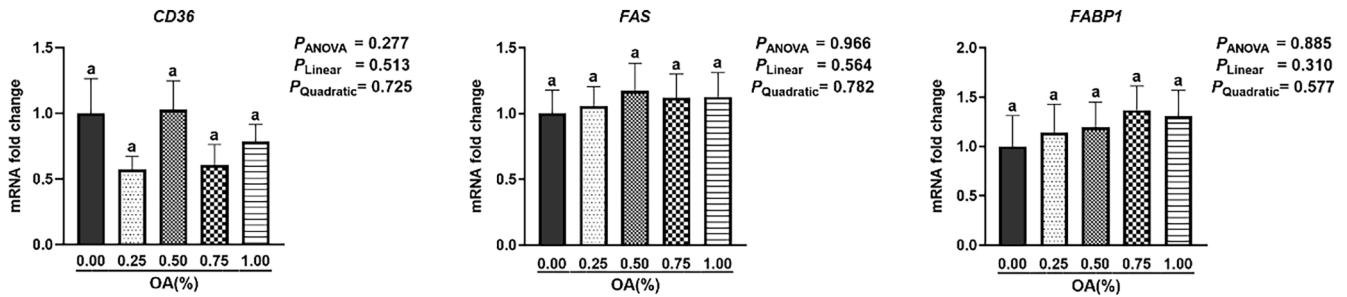


Fig. 2. The effects of diet supplemented with oleic acid on the expression of genes related to fatty acid transport of jejunal mucosa in Pekin ducks. CD36, cluster of differentiation 36; FAS, fatty acid synthase; FABP1, fatty acidbinding protein 1. Con,the basal diet; OA, the Con plus different levels of OA (0.25, 0.50, 0.75 and 1.00%). Data are means $\pm$ SEM represented by vertical bars or plot individual values(n=7).

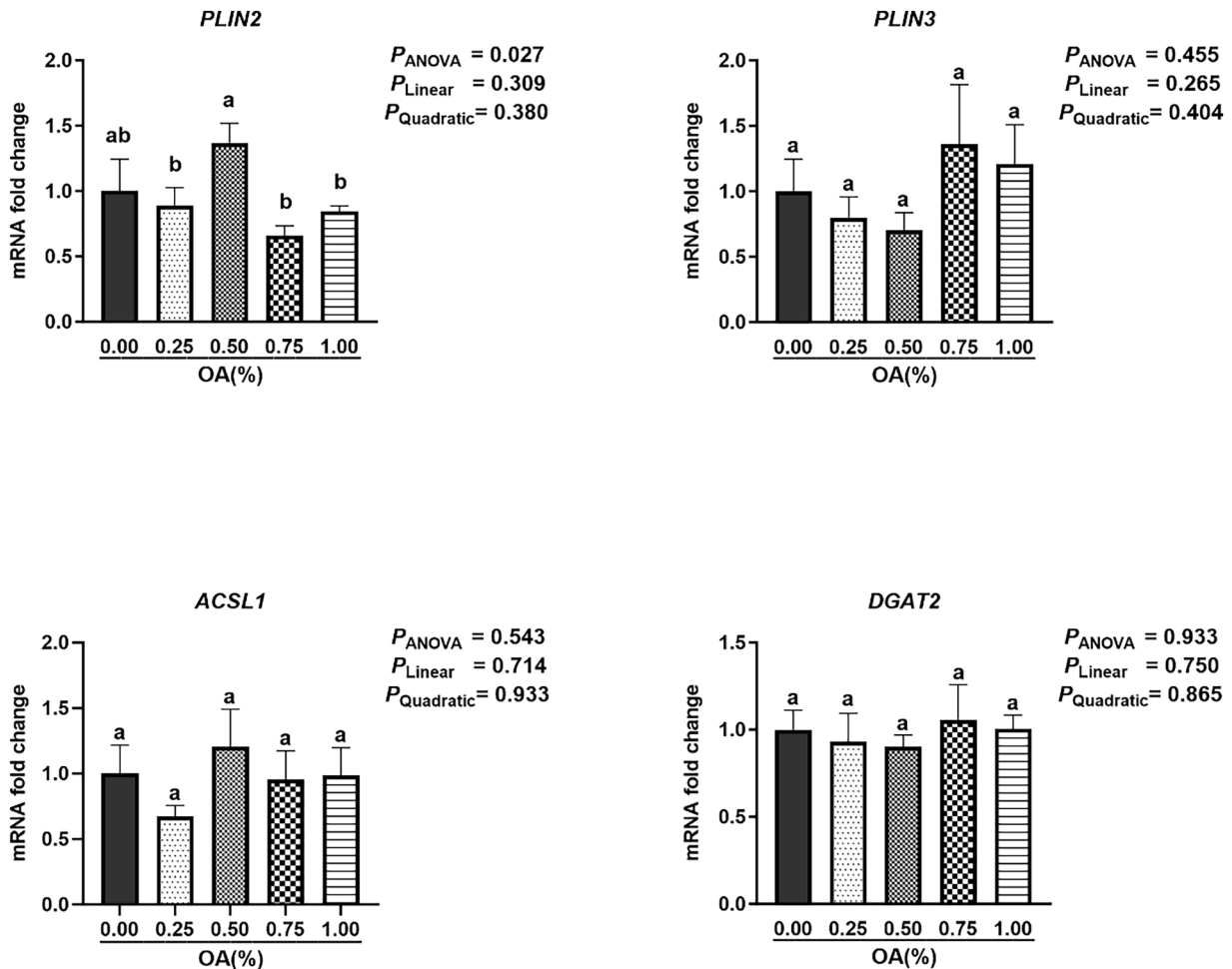


Fig. 3. The effects of diet supplemented with oleic acid on the expression of genes related to lipid droplet formation of jejunal mucosa in Pekin ducks. PLIN2, perilipin 2; PLIN3, perilipin 3; ACSL1, acyl-CoA synthetase long-chain family member 1; DGAT2, diacylglycerol o-acyltransferase 2. Con,the basal diet; OA, the Con plus different levels of OA (0.25, 0.50, 0.75 and 1.00%). Data are means $\pm$ SEM represented by vertical bars or plot individual values(n=7). a, b Means without a common superscript letter differ (p < 0.05).

(CD36, FABP1, and FAS) and LD formation-related genes (PLIN3, ACSL1, and DGAT2) in the jejunal mucosa ($P > 0.05$). However, the mRNA expression of the LD formation-related gene *PLIN2* was significantly lower in the 0.25% and 0.75% OA groups compared with the 0.50% OA group ($P < 0.05$). The mRNA expression of the LD degradation-related gene *PNPLA2* in the 0.25%, 0.50%, and 0.75% OA groups was down-regulated when compared with that in the control group ($P < 0.05$). The *LPL* mRNA levels in the OA-treated groups were lower than those in the control group ($P < 0.05$).

Discussion

The present study found that diets containing 9% palm oil with supplemented with different concentrations of OA had no significant effect on the growth performance of Pekin ducks aged from 14 to 42d. These results are similar to those observed by [Toomer et al. \(2020\)](#), who reported that the addition of 6% OA to a meat-type broiler chicken diet had no significant effect on BW or the feed conversion ratio (FCR). However, in the present study, dietary fat utilization in the 0.50% OA

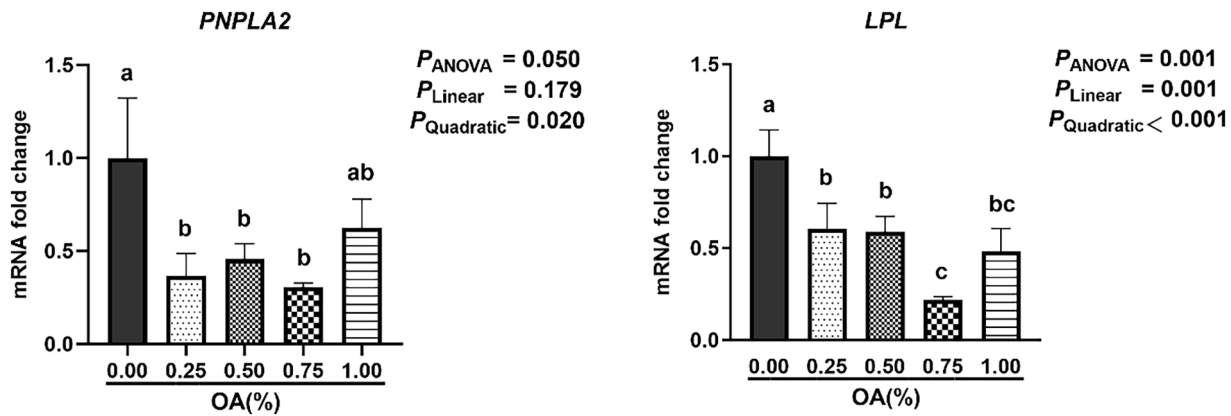


Fig. 4. The effects of diet supplemented with oleic acid on the expression of genes related to lipid droplet degradation of jejunal mucosa in Pekin ducks. PNPLA2, patatin like phospholipase domain containing 2; LPL, lipoprotein lipase. Con, the basal diet; OA, the Con plus different levels of OA (0.25, 0.50, 0.75 and 1.00%). Data are means $\pm$ SEM represented by vertical bars or plot individual values ($n=7$). a, b Means without a common superscript letter differ ($p < 0.05$).

group surpassed that of the control group by 3.16%. An earlier study revealed that a diet supplemented with a mixed FA containing 28% *cis*-9 C18:1 significantly improved fat utilization in ruminants, as opposed to the SFA-supplemented diet (Bai et al., 2023). This outcome was expected, as OA has been noted to enhance the digestibility of overall FAs (Prom and Lock, 2021). The reason may be that the amphiphilic property of OA increases its solubility in the intestine, which enhances the solubility of SFAs in micelles and facilitates their rapid absorption and re-esterification in intestinal epithelial cells (Krogdahl, 1985; Kouba and Mourot, 1998; Ravindran et al., 2016). The observed increase in dietary fat utilization with 0.50% OA supplementation, despite the absence of growth performance improvement, may be attributed to the primary function of oleic acid in regulating lipid metabolism within the organism. It is possible that an enhancement in slaughter performance occurs, necessitating further investigation for confirmation.

In the present study, serum TG levels in the 0.50% OA group were significantly lower than those in the 0.25% and 0.75% OA groups, representing the lowest levels among all treatment groups. Serum TG may originate from two sources: exogenous TG, which is formed through the digestion and absorption of dietary fats in the intestine, and endogenous TG, which is synthesized by the liver, adipose tissue, and small intestine from carbohydrates, fatty acids, or other precursor substances through *de novo* lipogenesis (Nguyen et al., 2008). Previous studies have reported that dietary UFAs reduce endogenous TG synthesis (Qi et al., 2008) and facilitate TG transport from the blood to tissues (Sanz et al., 2000), contributing to decreased serum TG levels.

Additionally, a negative correlation was observed between serum TBA levels and OA supplementation levels, both in a linear and quadratic manner. Serum LEP levels in the 0.50% and 1.00% OA groups were significantly higher than those in the control group. BAs, as amphiphilic cholesterol metabolites, facilitate lipid digestion and absorption by forming mixed micelles in the small intestine (Wahlström et al., 2016; Molinaro et al., 2018). Previous research has indicated that liver TBA levels decrease in *db/db* mice consuming diets rich in mono-unsaturated FAs (Huang et al., 2020), a finding that is consistent with the results of this study. Moreover, LEP suppresses lipid release into the bloodstream by downregulating apolipoproteins (Stan et al., 2001) and can reduce the ectopic accumulation of lipids by stimulating FA oxidation in the liver (Muioio et al., 1997; Lee et al., 2002; Minokoshi et al., 2002). These factors may lead to a decrease in serum TG levels in ducks fed a diet supplemented with 0.50% OA.

Other important factors may be related to intestinal lipid metabolism. After being digested and absorbed, dietary fats enter intestinal cells, where they are re-esterified into TAGs. Some of the re-esterified TAGs are assembled into chylomicrons and enter the metabolic circulation. Moreover, some re-esterified TAGs form cLDs (Ockner and

Manning, 1974). cLDs serve as intracellular energy reservoirs, which play a dynamic regulatory role in lipid metabolism (Greenberg and Coleman, 2011). Perilipins (PLINs) are now acknowledged as key players in cLD formation. In the intestine of zebrafish fed a high-fat diet, PLIN3 was rapidly recruited to LDs and subsequently replaced by PLIN2 (Wilson et al., 2021). These findings suggest that PLIN2 plays a critical role in stabilize TAG storage within cLDs. Additionally, PLIN3 was detected in cLDs isolated from the jejunal cells of mice administered high doses of olive oil (rich in OA) (D'Aquila et al., 2015). These results align with those of the present study. We detected the expression of *PLIN2* and *PLIN3* in the jejunal mucosa of Pekin ducks and found that the expression of *PLIN2* was significantly upregulated in the 0.50% OA group when compared with other OA groups. This phenomenon can be attributed to the mediating role of OA, which re-esterifies superfluous dietary fatty acids into TAGs, thereby facilitating the formation and storage of cLDs within the jejunal mucosa of Pekin ducks.

Enterocyte cLDs undergo catabolism through both neutral lipolysis and lipophagy. ATGL, which catalyzes the rate-limiting step in neutral lipolysis, is responsible for the initial step of TG breakdown into FA (Qi et al., 2024). HSL subsequently breaks down DAG, whereas MGL targets MAG, producing FA and glycerol (Zechner et al., 2017). In *C. elegans* enterocytes, TAG storage within cLDs is inversely correlated with ATGL activity (Lee et al., 2014). Intestine-specific HSL deficiency in mice promoted cholesteryl ester (CE) accumulation in the small intestine (Obrowsky et al., 2012). Mice with intestine-specific MGL over-expression exhibit downregulated intestinal MAG levels and are susceptible to diet-induced obesity (Chon et al., 2012). Peng et al. (2011) demonstrated that palmitic acid was converted mainly to active lipids (phospholipids and diacylglycerol) in the absence of OA, but to TAG in the presence of OA. Similarly, in this current study, dietary OA decreased the enzymatic activities of ATGL, HSL, and MGL, as well as the gene expression levels of *PNPLA2*, reducing the production of diacylglycerol and promoting the conversion of excess lipids into TAG stored in the enterocyte cLDs of Pekin ducks.

LPL is an acidic hydrolase that hydrolyzes TG, which leads to a decrease in blood lipid levels. The absence of LPL in mice leads to an accumulation of TAG and cholesteryl esters in various tissues, including the small intestine (Du et al., 1998; Du et al., 2001; Aquil et al., 2014). CES2 exhibits TAG hydrolase activity and regulates serum or tissue TG levels by modulating lipolysis and lipogenesis (Li et al., 2016). In this current study, the activities of LPL and CES2 in the OA groups, as well as the expression levels of the *LPL* gene, were upregulated compared with those in the control group. Linear and quadratic regression analyses revealed that LPL activity was directly correlated with the concentration of OA. These observations suggest that dietary OA not only regulates the degradation of enterocyte cLDs via suppression of lipolytic enzyme

activities, but also further promotes the formation of enterocyte cLDs by regulating the activity of lipophagy-related enzymes in Pekin ducks.

Conclusions

Dietary supplementation with OA, especially at a level of 0.50%, may modulate the lipid metabolic network through multidimensional mechanisms including: 1) reducing serum TG levels, 2) upregulating the expression of LD formation-related gene, 3) downregulating the activity of key enzymes and expression of LD degradation-related genes, and 4) converting excess dietary lipids into cLDs which are stored in the intestine. These results provide new insights into further nutritional regulation of lipid metabolism in meat ducks.

Declaration of competing interest

There are no conflicts of interest in this study. All authors have read and approved this version of the article, and due care has been taken to ensure the integrity of the work. Neither the entire paper nor any part of its content has been published or has been accepted elsewhere. It is not being submitted to any other journal.

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