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Inhibitory Effect of Prickly Ash (*Zanthoxylum bungeanum*) Seed Kernel Oil on Lipid Metabolism of Grass Carp (*Ctenopharyngodon idellus*) in High-Fat Diet

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Abstract: To investigate the effect of prickly ash (*Zanthoxylum bungeanum*) seed kernel oil (PASO) on the lipid metabolism of grass carp (*Ctenopharyngodon idellus*) under a high-fat diet, PASO were added into two lipid-level (4 g/kg and 8 g/kg) diets to form four isonitrogenous diets: soybean oil (SO), PASO, high-fat soybean oil (HSO), and high-fat prickly ash seed oil (HPASO). A total of 216 healthy grass carp (9.43 ± 0.82 g) were randomly divided into four groups and fed with the four diets, respectively, for 56 days. The result showed that the viscerosomatic index (VSI) and the content of the crude lipid in the hepatopancreas and muscle was significantly higher by oil levels ($p < 0.05$). The linolenic acid content in the body of the fish significantly increased in PASO and HPASO compared to that in SO and HSO ($p < 0.05$). The fatty acid composition of the hepatopancreas, intraperitoneal fat, and muscle in four dietary groups was significantly similar to the fatty acid composition in the diets ($p < 0.05$). More significant fat infiltration and nuclear translocation in the hepatopancreas of fish was found in the HSO group but was decreased in the HPASO group. The adipocyte size in the intraperitoneal fat tissue in the PASO group was significantly lower than that in the SO group ($p < 0.05$). The relative mRNA expression of the lipogenesis-related genes *ppar-γ*, *cebp-α*, and *srebp-1c* was significantly down-regulated in the PASO group compared with the SO group ($p < 0.05$), and the mRNA expression of lipolysis-related genes *ppar-α* and *cpt-1* were significantly up-regulated in the PASO group ($p < 0.05$). In conclusion, dietary PASO showed the function of reducing lipid accumulation in the fish. This reduction might be attributed to the inhibition of the lipogenesis-related genes and the stimulation of the lipolysis-related genes, which were probably modulated by the high content of linolenic acid in PASO.

Keywords: prickly ash seed kernel oil; growth performance; lipid metabolism; grass carp (*Ctenopharyngodon idellus*)

Key Contribution: This study is the first to evaluate the effect of prickly ash seed kernel oil (PASO) on the lipid metabolism of grass carp fed a high-fat diet. The results showed the dietary PASO's function of reducing lipid accumulation in the fish. The study provides valuable preliminary data for the commercial development and feasibility of PASO in artificial diets for grass carp.

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1. Introduction

Lipids are the source of energy and the essential fatty acids for fish [1–3]; therefore, high-fat diets are constantly used in aquaculture for greater growth of fish and a reduced cost of feeds [4–7]. However, long term use of high-fat diets was found to have negative impacts on the growth and health of fish [8–10], which included excessive fat accumulation, growth inhibition, and weak antioxidant properties and immunity [11–13]. High-fat diets were also found to induce mitochondrial dysfunction and inhibit β-oxidation of fatty acids [14,15].

A previous report showed that dietary intake of polyunsaturated fatty acid (PUFA), e.g., linoleic acid (LA), linolenic acid (LNA), and n-3 long-chain PUFAs (LC-PUFAs), etc., was associated with reduced lipid accumulation in the hepatopancreatic of fish [16–18], and the PUFA contributed to the inhibition of lipid synthesis by inhibiting the gene expression of lipogeneses and improving the gene expression of lipolysis [19,20].

Prickly ash (*Zanthoxylum bungeanum*) is the fruit of the Zanthoxylum plant in the Rutaceae family, which is widely cultivated in China, and there is a huge annual yield production of more than 500,000 tons [21]. The husk of the prickly ash seed (PAS) is commonly used as a spice in Chinese cuisine, while the kernel is often overlooked and discarded as waste. However, the PAS kernel contains a large amount of lipids, ranging from 27% to 31% [22]. PAS oil (PASO) is rich in unsaturated fatty acids, accounting for 70% of the total fatty acids, including 17.7~32.64% LA and 17.36~24.13% LNA, respectively, which are promising ingredients for fish feed [23,24]. A previous report showed that the levels of T-chol, TG, and LDL-C in the serum of mice [25] and the lipid level in the blood of rats [26] and hamsters [27] was reduced by oral administration of PASO, indicating that PASO may promote lipid metabolism and inhibit lipid accumulation in mammals, highlighting its potential as an oil source in feed for improving animal health and performance.

The grass carp (*Ctenopharyngodon idellus*) is a typically herbivorous fish and yields the highest production of freshwater fish in China [28]. In previous research, it was shown that the lipid requirement of grass carp is about 6% (60 g/k), and higher dietary lipid levels were found to cause excessive lipid accumulation and even injure the health of fish [29,30]. While the utilization of feeds with high lipid levels are common in the actual aquaculture of grass carp to gain higher production [31,32], the dietary use of PUFA to reduce lipid accumulation in grass carp has also been reported [33,34]. However, information about the utilization of PASO, with its richness in LA and LNA, to inhibit excessive accumulation of lipids in grass carp fed a high-fat diet is very scarce. Therefore, in the present study, PASO was added in normal and high-fat diets of grass carp, respectively, to form four diets, and the grass carp were fed the diets, respectively, for 56 days; then, the fish were sampled, and the growth and lipid metabolism were determined.

2. Materials and Methods

2.1. Experimental Diets

The prickly ash seed oil (PASO) was obtained from the pressing of prickly ash seed purchased from Hancheng, Shaanxi. Feed resources such as fish meal and soybean meal, etc., were purchased from Shaanxi Huaqin Agriculture and Animal Husbandry Technology Co., Ltd. (Yangling, Shaanxi, China). PASO was added into two diets at normal (4 g/kg)- and high (8 g/kg)-lipid levels, respectively, to form four isonitrogenous feeds: the soybean oil (SO), PASO, high-fat soybean oil (HSO), and high-fat prickly ash seed oil (HPASO) groups. All the ingredients were crushed and mixed thoroughly to form a dough, which was pressed into granules with a diameter of 2 mm. The experimental diets were stored at −20 °C after air drying. The formula and the proximate composition of the diets are given in Table 1.

Table 1. The formulation and proximate composition of the diets.

Ingredients (g/kg)	SO	PASO	HSO	HPASO
Fish meal	40	40	40	40
Soybean meal	198	198	198	198
Rapeseed meal	20	20	150	150
Cottonseed meal	297	297	252	252
Whole wheat flour	362	362	176	176
Rice bran	22	220	1020	1020

Table 1. *Cont.*

Ingredients (g/kg)	SO	PASO	HSO	HPASO
Soybean oil	210	00	420	00
PASO	0	210	0	420
Bentonite	100	100	100	100
$Ca(H_2PO_4)$	200	200	200	200
Premix *	100	100	100	100
VC	0.002	0.002	0.002	0.002
Total	1000	1000	1000	1000
Proximate composition (%) **				
Crude protein	33.14	33.26	33.17	33.77
Crude lipid	4.11	3.80	8.28	8.07
Ash	7.66	7.67	8.77	8.64
Moisture	8.43	8.66	8.09	8.50
Gross energy (MJ/kg) ***	19.02	18.95	19.80	19.81

* Fish premix, selected from Beijing Yinhuier Biological Technology Co., Ltd., Beijing, China. The content of premix per kilogram of fish is as follows: vitamin A 810,000 IU, vitamin D3 198,000 IU, vitamin E 4500 mg, vitamin K31,440 mg, vitamin B1900 mg, vitamin B21,350 mg, vitamin B6830 mg, vitamin C 13,000 mg, vitamin B3110 mg, inositol 12,000 mg, iron 12,000 mg, copper 400 mg, zinc 3000 mg, manganese 1500 mg, magnesium 60,000 mg, cobalt 40 mg, iodine 100 mg, and selenium 30 mg. ** Measured value; *** theoretical value.

2.2. Fish and Feeding Management

The total of 216 healthy grass carp (mean weight 9.43 ± 0.82 g), provided by Ankang Aquatic Experiment Demonstration Station of Northwest A&F University (Shanxi, China), were randomly and evenly assigned into four groups (triplicate per group) and cultured in the 12 net cages (1.5 m × 1.0 m × 1.0 m) erected in outdoor incubation tanks (4.75 m × 3.65 m × 0.8 m); then, they were fed with the four diets mentioned above three times a day (08:30, 12:30, and 16:30) by hand to apparent satiation. The fish were kept under natural light conditions, and micro-flowing water circulation was used 24 h a day during the experimental period, and the water temperature, dissolved oxygen, pH, and ammonia content were 28.0 ± 2.1 °C, 7.5 ± 1.54 mg/L, 7.8 ± 0.20, and 0.05 ± 0.01 mg/L, respectively. The feeding period was 56 days.

2.3. Sample Collection

Prior to the sampling collection, the fish were fasted for 24 h; then. they were anesthetized using the 100 mg/L MS-222 (tricaine isophthalic acid ethyl methanesulfonate) to obtain the weight and length of each fish. The weight of the viscera, hepatopancreas, and abdominal fat of the six fish per cage were obtained, and the hepatopancreas and abdominal fat were sampled and stored in 4% paraformaldehyde solution. Then, samples of the hepatopancreas, abdominal fat, and muscle were taken from another six fish to measure proximate composition. Finally, the hepatopancreas, abdominal fat, and muscle of the remaining six fish were sampled, quickly frozen in liquid nitrogen, and then stored at −80 °C for biochemical analyses, fatty acid profile, and gene expressions.

Growth performance and various biological parameters were calculated using the following formulas:

Survival rate (SR, %) = (final number of fish/initial number of fish) × 100.
Specific growth rate (SGR, %/d) =100 × [ln final body weight (g) − ln initial body weight (g)]/days.
Feed conversion ratio (FCR, g/g) = feed provided (g)/body weight gain (g)].
Condition factor (CF, g/cm^3) = 100 × [body weight (g)/body length (cm)^3].
Hepatosomatic index (HSI, %) =100 × [hepatopancreas weight (g)/body weight (g)].
Viscerosomatic index (VSI, %) = 100 × [weight of viscera (g)/body weight (g)].
Intraperitoneal fat index (IPFI, %) = 100 × [intraperitoneal fat weight (g)/body weight (g)].

2.4. Proximate Composition Analysis

The contents of crude protein, crude fat, moisture, and ash in the diets, whole fish body, hepatopancreas, and the muscle tissues were analyzed by the methods of AOAC [35], where the Kjeldahl method, Soxhlet extraction method, 70 degrees constant weight drying method and 550 °C muffle furnace burning method were used, respectively.

2.5. Fatty Acid Composition Analysis

Firstly, the 0.3~0.5 g tissue sample was homogenized in 5 mL chloroform/methanol (2:1 *v*/*v*) with a high-speed disperser (XHF-D, SCIENIZ®, Ningbo, China), and the supernatant was obtained by filtering the solid residue after standing for 1–2 h. Then, 4 mL of deionized water was added to the supernatant sample, mixed, and then centrifuged at 3000 rpm for 5 min. The upper layer was sucked out, and the bottom layer was drained by nitrogen sweeping method in a water bath (40 °C) to obtain the pure lipids. Subsequently, 1 mL of chromatographically pure hexane was added to dissolve the lipids, and then, 1 mL of potassium hydroxide methanol (0.4 M) was added for 30 min for methyl esterification. Finally, 2 mL of deionized water was added and centrifuged to extract the supernatant for determination on a gas chromatograph (Agilent 7820A, Agilent Technologies, Santa Clara, CA, USA). Each fatty acid was identified against a known standard (47015-U, Sigma-Aldrich, Inc., St. Louis, MO, USA). The results were calculated by the area normalization method and are presented as a percentage of each fatty acid to total fatty acids. The fatty acid composition of the diets is given in Table 2.

Table 2. The fatty acid composition of the diets (%).

Fatty Acids	SO	PASO	HSO	HPASO
14:0	1.14	2.56	0.58	0.71
16:0	14.78	18.10	12.66	14.79
18:0	5.49	3.00	4.02	2.59
Σ SFA	21.42	23.66	17.26	18.09
16:1n-7	0.93	7.09	0.58	6.44
18:1n-9	21.66	25.87	27.77	34.63
Σ MUFA	22.60	32.96	28.36	41.07
18:2n-6	42.92	27.15	42.55	27.27
20:4n-6	1.94	2.30	2.48	2.58
22:4n-6	1.70	2.79	1.05	0.43
Σ n-6PUFA	46.56	32.24	46.08	30.29
18:3n-3	5.66	7.45	6.27	8.88
20:5n-3	2.55	2.68	1.43	1.05
22:6n-3	1.22	1.01	0.61	0.63
Σ n-3PUFA	9.43	11.14	8.31	10.56

Table 2. *Cont.*

Fatty Acids	SO	PASO	HSO	HPASO
n-3/n-6 PUFA	0.20	0.35	0.18	0.35
LA/LNA	0.13	0.27	0.15	0.33

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, poly-unsaturated fatty acid; LA, linoleic acid; LNA, α-linolenic acid. (Abbreviations are standard throughout.)

2.6. Histological Observation

The fixed samples were washed in running water for 12 h. According to the standard procedures, the samples were dehydrated in ethanol with gradient concentrations, soaked in xylene, and embedded in paraffin. A 5 μm thick paraffin section was successively cut using a slicer (RM2235, Leica, Germany) and mounted on a slide and stained with hematoxylin and eosin (H&E). All histological procedures were performed at the Pathology Laboratory of the Yangling Demonstration Zone Hospital (Yangling, Shaanxi, China). Images were taken using a forward microscope (Leica Biosystems, Germany). Adipocyte area per image was quantified using Photoshop (Adobe, San Jose, CA, USA), and the mean adipocyte area per group was calculated by five non-overlapping images of adipose tissue.

2.7. Gene Expression Analysis

Total RNA extraction and reverse transcription from tissues were performed using methods common to our laboratory. The CFX96 Real-Time Quantitative PCR Detection System (Bio-Rad, Hercules, CA, USA). was used for real-time quantitative PCR determination of gene expression. According to the kit protocol, the 20 μL reaction system contained 0.6 μL primers, 1 μL diluted cDNA, 10 μL 2× SYBR® Premix Ex TaqTM II, and 7.8 μL sterile non-enzymatic water. The qPCR reaction conditions were followed: an initial activation step at 95 °C for 30 s, followed by 40 cycles (95 °C for 15 s, 60 °C for 15 s). The CT values of the target genes and the housekeeping gene (β-actin) were obtained using the amplification curves after PCR reactions, and the relative expression of target gene in each group was calculated according to the formula $2^{-\Delta\Delta CT}$. The PCR primer sequences of each gene are shown in Table 3.

Table 3. Nucleotide sequences of the primers.

Genes		Sequences of Primers (5′~3′)	Accession Number
Lipogenesis-related genes	*ppar-γ*	Forward: GATGGTTGGCATGTCACAC Reverse: TTCCTGACAGTATGGCTC	FJ849064
	cebp-α	Forward: ACCCACATACCACCACTCTCAACA Reverse: TTTCCCTCGATCGCCCATCTTCAT	NM199529
	srebp-1c	Forward: GGATTGAGGTGAGCCGACAT Reverse: TGAGGAAAGCCATTGACTACATT	KJ162572
Lipolysis-related genes	*ppar-α*	Forward: AGTGATGGTGGTCTTCAGCTCCGA Reverse: AGTGTCGGACTTCAGCTCCAAAGA	FJ623265
	cpt-1	Forward: CATGGCATTGACCGCCATCTCTTT Reverse: AGAGTTTCCAAGGCTCTGACAGCA	KJ816749
	ucp-1	Forward: CGTGGTTTGTGGAAAGG Reverse: GCTCCAAATGCAGATGTG	AY689125
Housekeeping gene	*β-actin*	Forward: CGTGACATCAAGGAGAAG Reverse: GAGTTGAAGGTGGTCTCAT	DQ211096

ppar-γ, peroxisome proliferator-activated receptor-γ; *cebp-α*, *ccaat* enhancer binding protein-α; *srebp-1c*, sterol regulatory element binding transcription factor 1c; *ppar-α*, peroxisome proliferator-activated receptor-α; *cpt-1*, carnitine palmitoyl transferase 1; *ucp-1*, uncoupling protein 1.

2.8. Data Analysis

Data are presented as mean ± S.D. (standard deviation). Experimental data were analyzed by two-way ANOVA and tested for homogeneity of variance by Levene's test, followed

by Duncan's multiple comparisons to determine differences between groups. The non-nested statistical analyses were performed using SPSS 21.0 for Windows Software (SPSS, Chicago, IL, USA). The significance difference was determined at a probability of 5% ($p < 0.05$). The Pearson correlation coefficients (PCCs) were calculated by the CORREL function with reference to Tian's study [36], which shows the tissue–diet correlation coefficients.

3. Results

3.1. Growth Performance and Biological Parameters

Table 4 shows that the FBW, SR, SGR, FCR, CF, HSI, and IPFI of grass carp were not significantly affected by oil levels and oil sources ($p > 0.05$). The VSI of the HSO and HPASO groups was significantly higher than that of the SO group ($p < 0.05$) and was significantly affected by the oil level, and the interaction between the oil level and oil source had an impact on VSI ($p < 0.05$).

Table 4. Effects of PASO on growth performance and biological indices of grass carp ($n = 3$).

Parameters	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
IBW (g)	9.43 ± 0.82	9.43 ± 0.82	9.43 ± 0.82	9.43 ± 0.82	ns	ns	ns
FBW (g)	33.50 ± 2.32	33.33 ± 1.57	35.07 ± 2.74	35.45 ± 2.62	ns	ns	ns
SR (%)	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	ns	ns	ns
SGR (%/d)	2.43 ± 0.16	2.25 ± 0.10	2.35 ± 0.19	2.36 ± 0.16	ns	ns	ns
FCR	1.29 ± 0.04	1.28 ± 0.05	1.29 ± 0.08	1.23 ± 0.06	ns	ns	ns
CF (g/cm^3)	1.87 ± 0.03	1.83 ± 0.02	1.92 ± 0.05	1.90 ± 0.02	ns	ns	ns
HSI (%)	2.29 ± 0.14	2.30 ± 0.14	2.17 ± 0.16	2.27 ± 0.15	ns	ns	ns
VSI (%)	12.39 ± 0.80 ^{b}	13.30 ± 0.18 ^{ab}	14.33 ± 0.96 ^{a}	13.78 ± 0.40 ^{a}	*	ns	*
IPFI (%)	1.85 ± 0.49	1.94 ± 0.17	2.05 ± 0.13	2.20 ± 0.62	ns	ns	ns

Data represent mean ± standard deviations. Different superscripts in the same row denote significant differences. ns, not significant; * $p < 0.05$.

3.2. Proximate Composition of the Hepatopancreas and Muscle

As shown in Table 5, there was a significant interaction between oil levels and oil sources, which impacted the hepatopancreatic crude protein level ($p < 0.05$). Both the hepatopancreas and muscle crude lipid levels were significantly affected by oil levels ($p < 0.05$). And there were no significant differences in hepatopancreas moisture and ash and muscle crude fat, moisture, and ash ($p > 0.05$).

Table 5. Effects of PASO on proximate composition of the hepatopancreas and muscle of grass carp (%, air-dried basis, $n = 3$).

Parameters	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
			Hepatopancreas				
Crude protein	30.73 ± 2.02	27.36 ± 0.41	29.32 ± 2.14	30.92 ± 0.98	ns	ns	*
Crude lipid	40.81 ± 1.18 ^{b}	40.28 ± 0.36 ^{b}	48.53 ± 2.23 ^{a}	47.12 ± 1.05 ^{a}	*	ns	ns
Moisture	7.34 ± 0.15	7.54 ± 1.22	7.46 ± 1.06	7.90 ± 0.95	ns	ns	ns
Ash	3.07 ± 0.01	3.02 ± 0.01	2.65 ± 0.01	3.12 ± 0.01	ns	ns	ns
			Muscle				
Crude protein	74.20 ± 3.59	74.81 ± 3.12	76.83 ± 3.53	74.06 ± 2.52	ns	ns	ns
Crude lipid	8.11 ± 0.66 ^{b}	7.75 ± 1.38 ^{b}	10.81 ± 1.42 ^{a}	10.50 ± 1.28 ^{a}	*	ns	ns

Table 5. *Cont.*

Parameters	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
Moisture	15.48 ± 0.64	15.31 ± 0.79	15.10 ± 1.16	14.90 ± 0.89	ns	ns	ns
Ash	6.04 ± 0.07	5.97 ± 0.12	6.05 ± 0.19	6.00 ± 0.10	ns	ns	ns

Data represent mean ± standard deviations. a, b: different superscripts in the same row denote significant differences. ns, not significant; * $p < 0.05$.

3.3. Fatty Acid Composition

The fatty acid composition of the hepatopancreas, intraperitoneal fat, and muscle is presented in Tables 6–8, respectively. The oil levels significantly increased the LA, LNA, and n-3PUFA content of hepatopancreas, abdominal fat, and muscle ($p < 0.05$). PASO significantly elevated 16:1n-7 in the hepatopancreas, abdominal fat, and muscle as well as LNA in the abdominal fat. Additionally, in the hepatopancreas, abdominal fat, and muscle, the correlation between the HPASO group and dietary fatty acid composition was significantly higher than that with the other groups ($p < 0.05$).

Table 6. Effects of PASO on fatty acid composition of hepatopancreas in grass carp (%, $n = 3$).

Fatty Acid	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
14:0	2.65 ± 0.22 ^{a}	2.72 ± 0.26 ^{a}	2.22 ± 0.30 ^{b}	2.18 ± 0.21 ^{b}	*	ns	ns
16:0	20.59 ± 0.36 ^{a}	20.13 ± 0.30 ^{a}	18.51 ± 0.30 ^{b}	18.07 ± 1.12 ^{b}	*	ns	ns
18:0	9.80 ± 0.64 ^{a}	8.97 ± 0.44 ^{ab}	8.83 ± 0.92 ^{ab}	8.12 ± 1.00 ^{b}	*	ns	ns
Σ SFA	33.03 ± 0.87 ^{a}	31.82 ± 0.49 ^{a}	29.56 ± 1.05 ^{b}	28.37 ± 1.88 ^{b}	*	ns	ns
16:1n-7	8.22 ± 0.72 ^{b}	10.05 ± 0.55 ^{a}	6.68 ± 1.11 ^{c}	7.50 ± 1.16 ^{bc}	*	*	ns
18:1n-9	47.33 ± 1.42	49.88 ± 0.94	48.09 ± 2.20	49.12 ± 2.93	ns	ns	ns
24:1	0.74 ± 0.27	0.54 ± 0.16	0.97 ± 0.16	0.78 ± 0.30	ns	ns	ns
Σ MUFA	56.29 ± 1.93	60.47 ± 1.28	55.75 ± 3.02	56.40 ± 3.58	ns	ns	ns
18:2n-6	5.98 ± 2.31 ^{a}	3.32 ± 0.82 ^{b}	8.43 ± 3.05 ^{a}	8.31 ± 1.89 ^{a}	*	ns	ns
20:4n-6	1.54 ± 0.49 ^{b}	1.36 ± 0.49 ^{b}	2.31 ± 0.45 ^{a}	1.99 ± 0.68 ^{ab}	*	ns	ns
Σ n-6PUFA	7.53 ± 1.95 ^{b}	4.67 ± 0.78 ^{c}	10.74 ± 3.31 ^{a}	10.30 ± 2.36 ^{a}	*	ns	ns
18:3n-3	1.74 ± 0.41 ^{c}	1.85 ± 0.21 ^{bc}	2.27 ± 0.37 ^{ab}	2.61 ± 0.47 ^{a}	*	ns	ns
20:5n-3	0.33 ± 0.12	0.23 ± 0.07	0.35 ± 0.11	0.31 ± 0.13	ns	ns	ns
22:6n-3	0.98 ± 0.29	1.06 ± 0.45	1.33 ± 0.16	1.23 ± 0.33	ns	ns	ns
Σ n-3PUFA	3.16 ± 0.16 ^{b}	3.04 ± 0.53 ^{b}	3.95 ± 0.38 ^{a}	4.15 ± 0.72 ^{a}	*	ns	ns
n-3/n-6PUFA	0.44 ± 0.11 ^{b}	0.65 ± 0.08 ^{a}	0.40 ± 0.13 ^{b}	0.41 ± 0.08 ^{b}	*	ns	ns
PCCs	0.36 ± 0.03 ^{d}	0.66 ± 0.01 ^{b}	0.51 ± 0.04 ^{c}	0.82 ± 0.01 ^{a}	*	*	ns

Data represent mean ± standard deviations. a, b, c: different superscripts in the same row denote significant differences. ns, not significant; * $p < 0.05$.

Table 7. Effects of PASO on fatty acid composition of intraperitoneal fat in grass carp (%, n = 3).

Fatty Acid	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
14:0	1.94 ± 0.08 ^{a}	1.90 ± 0.06 ^{a}	1.26 ± 0.10 ^{b}	1.33 ± 0.02 ^{b}	*	ns	ns
16:0	19.03 ± 0.08 ^{a}	19.30 ± 0.44 ^{a}	15.65 ± 0.97 ^{b}	15.84 ± 0.24 ^{b}	*	ns	ns
18:0	5.97 ± 0.18 ^{a}	5.83 ± 0.25 ^{a}	3.99 ± 0.23 ^{b}	3.83 ± 0.25 ^{b}	*	ns	ns
Σ SFA	26.95 ± 0.16 ^{a}	26.24 ± 0.45 ^{a}	20.90 ± 0.91 ^{b}	21.00 ± 0.27 ^{b}	*	ns	ns
16:1n-7	6.56 ± 0.45 ^{ab}	9.03 ± 0.35 ^{a}	5.37 ± 2.34 ^{b}	6.86 ± 1.94 ^{ab}	ns	*	ns
18:1n-9	38.62 ± 1.45	40.74 ± 0.61	39.10 ± 3.13	39.49 ± 2.38	ns	ns	ns
22:1n-9	0.78 ± 0.03	0.80 ± 0.03	0.84 ± 0.21	0.87 ± 0.16	ns	ns	ns
Σ MUFA	45.96 ± 1.71	50.37 ± 0.48	45.42 ± 5.24	47.22 ± 4.08	ns	ns	ns
18:2n-6	21.27 ± 1.12 ^{ab}	17.07 ± 0.91 ^{b}	26.63 ± 5.78 ^{a}	24.02 ± 4.54 ^{a}	*	ns	ns
20:4n-6	0.81 ± 0.12	0.67 ± 0.05	0.93 ± 0.33	0.92 ± 0.15	ns	ns	ns
Σ n-6PUFA	22.08 ± 1.22 ^{ab}	17.74 ± 0.89 ^{b}	27.56 ± 6.10 ^{a}	24.93 ± 4.66 ^{a}	*	ns	ns
18:3n-3	4.11 ± 0.51 ^{c}	4.80 ± 0.17 ^{b}	5.28 ± 0.39 ^{ab}	5.92 ± 0.80 ^{a}	*	*	ns
20:5n-3	0.50 ± 0.10	0.49 ± 0.08	0.44 ± 0.04	0.49 ± 0.05	ns	ns	ns
22:6n-3	0.40 ± 0.06	0.37 ± 0.04	0.39 ± 0.07	0.44 ± 0.09	ns	ns	ns
Σ n-3PUFA	5.01 ± 0.63 ^{c}	5.66 ± 0.28 ^{bc}	6.11 ± 0.39 ^{ab}	6.85 ± 0.85 ^{a}	*	ns	ns
n-3/n-6PUFA	0.23 ± 0.02 ^{b}	0.32 ± 0.02 ^{a}	0.23 ± 0.06 ^{b}	0.28 ± 0.07 ^{ab}	ns	*	ns
PCCs	0.68 ± 0.01 ^{a}	0.86 ± 0.01 ^{b}	0.84 ± 0.04 ^{b}	0.98 ± 0.01 ^{a}	*	*	ns

Data represent mean ± standard deviations. a, b, c: different superscripts in the same row denote significant differences. ns, not significant; * $p < 0.05$.

Table 8. Effects of PASO on fatty acid of muscle in grass carp (%, n = 3).

Fatty Acid	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
14:0	1.10 ± 0.07 ^{a}	1.03 ± 0.16 ^{ab}	0.93 ± 0.07 ^{bc}	0.82 ± 0.12 ^{c}	*	ns	ns
16:0	22.82 ± 0.72 ^{a}	22.25 ± 0.25 ^{a}	20.90 ± 1.00 ^{b}	19.79 ± 1.62 ^{b}	*	ns	ns
18:0	8.36 ± 0.67	7.89 ± 0.90	7.86 ± 0.56	7.08 ± 1.13	ns	ns	ns
Σ SFA	32.28 ± 1.31 ^{a}	31.17 ± 0.97 ^{ab}	29.57 ± 1.25 ^{bc}	27.80 ± 2.63 ^{c}	*	ns	ns
16:1n-7	5.62 ± 0.38 ^{b}	6.95 ± 0.56 ^{a}	3.77 ± 0.99 ^{c}	4.60 ± 0.91 ^{c}	*	*	ns
18:1n-9	21.50 ± 1.90	23.83 ± 1.91	22.56 ± 2.12	26.52 ± 5.40	ns	ns	ns
22:1n-9	2.08 ± 0.14	1.95 ± 0.18	2.44 ± 0.26	2.04 ± 0.53	ns	ns	ns
24:1	4.07 ± 0.46	3.33 ± 0.31	3.88 ± 0.74	3.30 ± 1.16	ns	ns	ns
Σ MUFA	33.28 ± 1.69 ^{ab}	36.06 ± 1.90 ^{ab}	32.65 ± 2.00 ^{b}	36.45 ± 4.19 ^{a}	ns	*	ns
18:2n-6	11.27 ± 0.74 ^{b}	9.63 ± 0.90 ^{b}	15.27 ± 2.42 ^{a}	16.02 ± 4.01 ^{a}	*	ns	ns
20:3n-6	1.43 ± 0.24 ^{b}	2.00 ± 0.35 ^{a}	0.94 ± 0.25 ^{c}	0.71 ± 0.06 ^{c}	*	ns	*
20:4n-6	7.26 ± 0.65	6.60 ± 0.34	7.62 ± 1.26	5.95 ± 1.82	ns	ns	ns
Σ n-6PUFA	19.95 ± 0.94 ^{bc}	18.23 ± 0.67 ^{c}	23.84 ± 3.11 ^{a}	22.68 ± 3.34 ^{ab}	*	ns	ns
18:3n-3	2.03 ± 0.34 ^{b}	2.26 ± 0.31 ^{b}	2.56 ± 0.41 ^{b}	3.16 ± 0.64 ^{a}	*	ns	ns
20:3n-3	2.85 ± 0.61	2.38 ± 0.73	2.08 ± 0.44	1.92 ± 0.65	ns	ns	ns
20:5n-3	1.63 ± 0.45	1.91 ± 0.29	1.75 ± 0.22	1.49 ± 0.57	ns	ns	ns
22:6n-3	7.98 ± 1.05	7.99 ± 1.06	7.54 ± 0.51	6.50 ± 2.55	ns	ns	ns

Table 8. *Cont.*

Fatty Acid	Diets				Two-Way ANOVA		
	SO	PASO	HSO	HPASO	Oil Levels	Oil Sources	Interaction
Σ n-3PUFA	14.49 ± 1.43	14.54 ± 1.67	13.94 ± 1.08	13.06 ± 3.28	ns	ns	ns
n-3/n-6PUFA	0.73 ± 0.10 ^{ab}	0.80 ± 0.12 ^{a}	0.60 ± 0.13 ^{b}	0.60 ± 0.20 ^{b}	*	ns	ns
PCCs	0.59 ± 0.00	0.82 ± 0.00	0.72 ± 0.03	0.91 ± 0.02	*	*	*

Data represent mean ± standard deviations. a, b, c: different superscripts in the same row denote significant differences. ns, not significant; * $p < 0.05$.

3.4. Histological Observation

The cell membranes of the hepatopancreas in the four groups were intact (Figure 1), while a higher degree of vacuolization and lipid droplets was found in the HSO group, and a lower degree of vacuolization and lipid droplets was found in the PASO and HPASO groups, respectively.

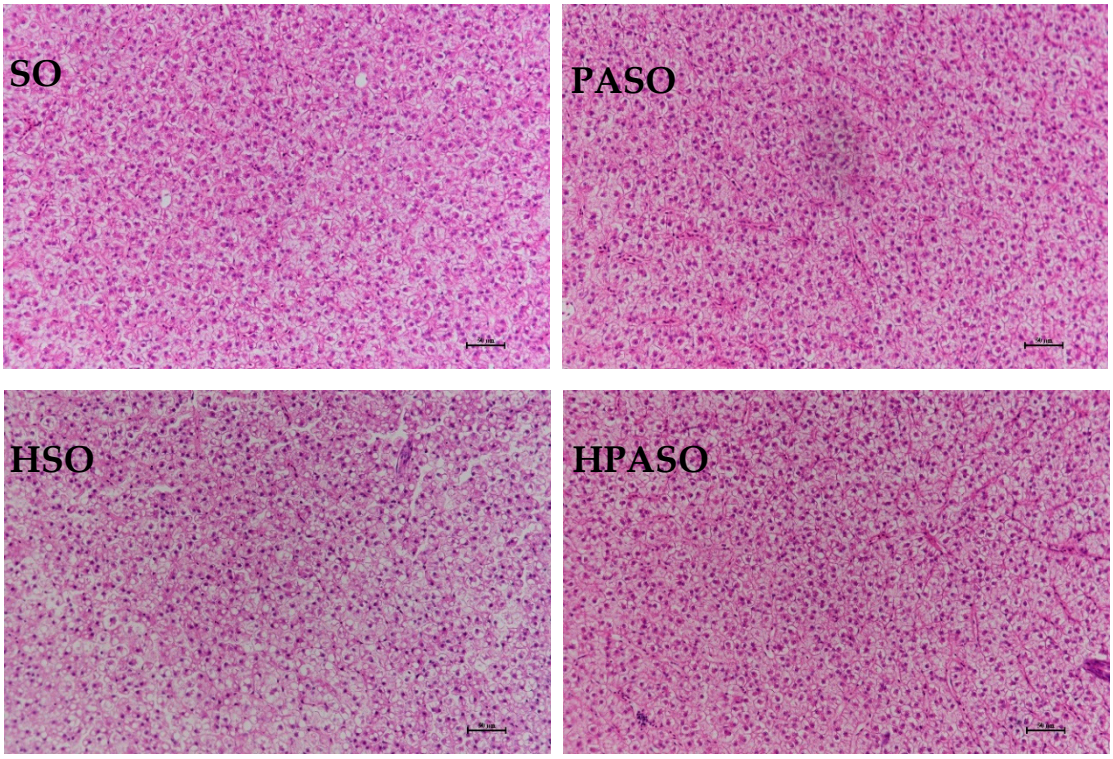

Figure 1. Hepatopancreas of the grass carp. (n = 3).

The cell membranes of the adipocytes in the abdominal adipose tissue were intact, and the cells were arranged regularly in the four dietary groups (Figure 2a). The adipocyte size in the PASO group was significantly lower than that in the SO group ($p < 0.05$; Figure 2b), while the adipocyte size was not significantly different between the HSO and HPASO groups ($p > 0.05$, Figure 2b). The frequency distribution of the greatest adipocyte size in the four dietary groups was within the range of 2000~5999 μm^2, which was above 10% (Figure 2c), and there was no significant difference among the four groups ($p > 0.05$, Figure 2c).

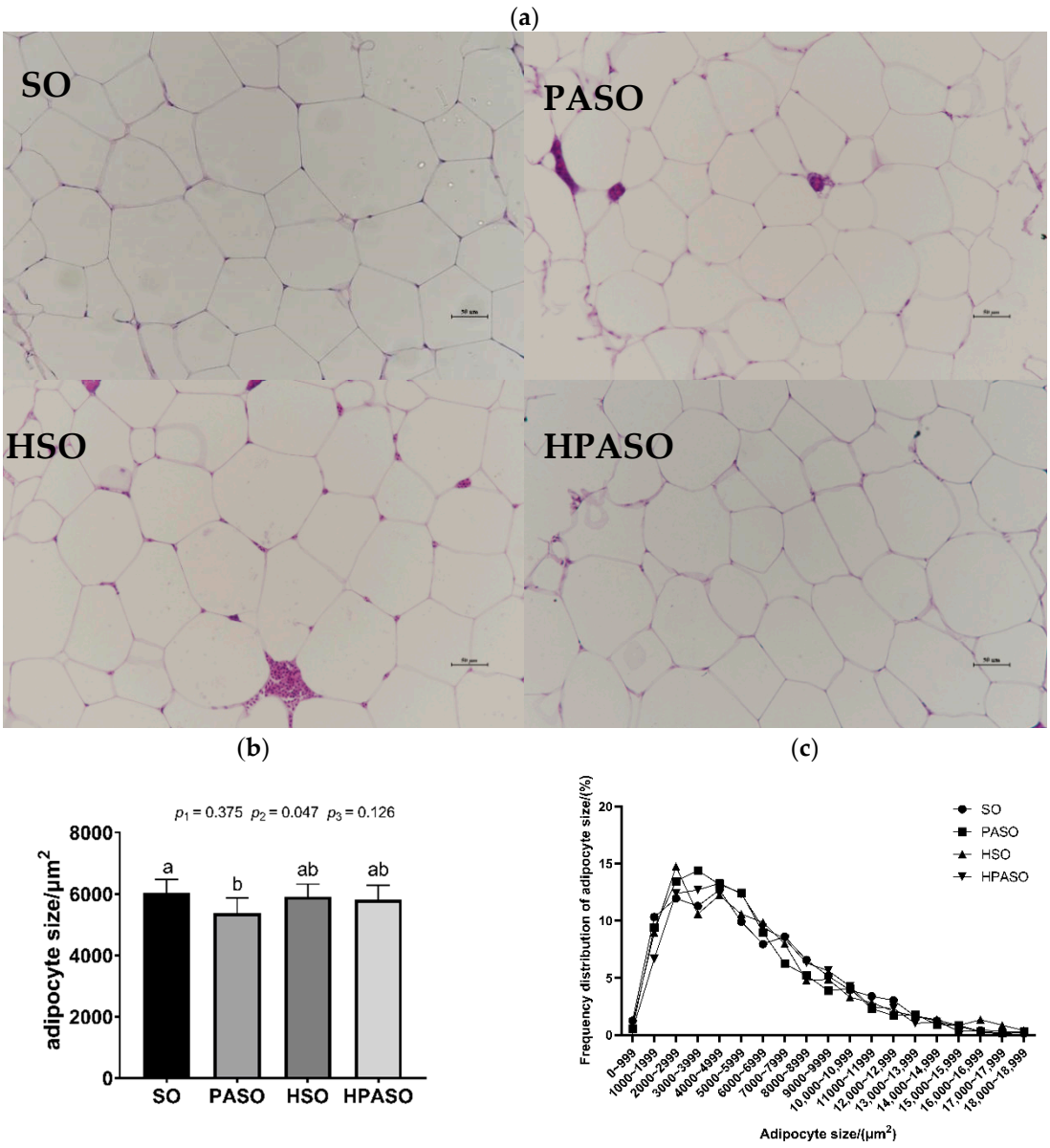

Figure 2. Adipocyte of the abdominal fat in grass carp. (**a**) Morphology of the abdominal fat tissues (HE stains, 10 × 20 times). (**b**) Adipocyte size of abdominal fat tissues (μm^2). (**c**) Frequency distribution of the adipocyte size. Different lowercase letters in the figure indicate significant differences. p_1, the effect of oil levels on data; p_2, the effect of oil sources on data; p_3, effect of the interaction between oil levels and oil sources on the data. $p < 0.05$ means significant difference. (%, $n = 3$).

3.5. Relative Expression of Genes Related to Lipid Metabolism

Figure 3 shows that in a normal-lipid-level diet, the mRNA relative expression of *ppar-γ*, *cebp-α*, and *srebp-1c*, being lipid-synthesis-related genes, significantly decreased in the PASO group compared with that in the SO group ($p < 0.05$), while in the high-lipid-level diet, the mRNA expression levels of *cebp-α* and *srebp-1c* (but not *ppar-γ*) were not significantly different between the HSO and HPASO groups ($p > 0.05$). The results of the two-way ANOVA showed that *ppar-γ*, *cebp-α*, and *srebp-1c* were affected by the interaction between oil sources and oil levels; in addition, PASO had a significant effect on *srebp-1c*.

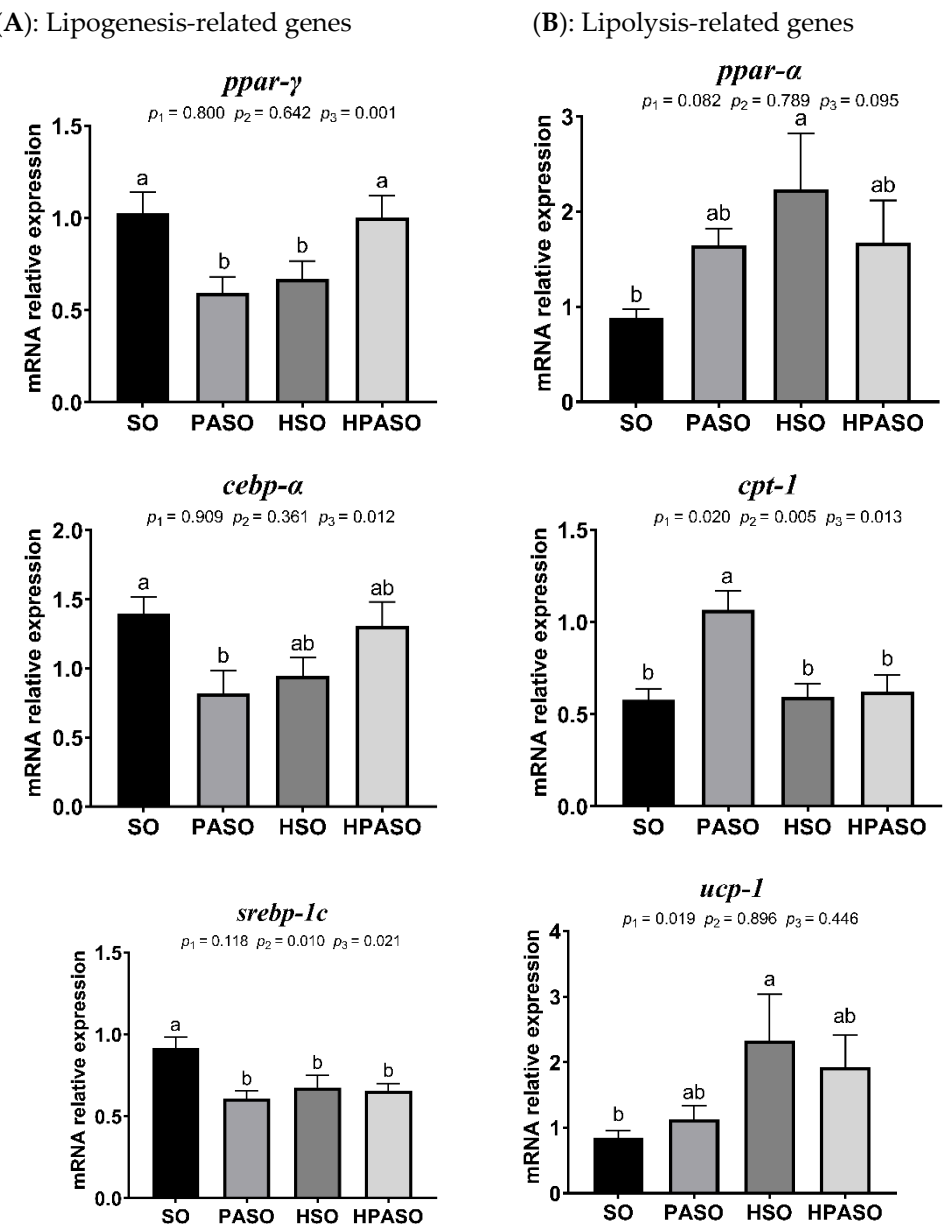

Figure 3. Effects of prickly ash seed oil on the lipid-metabolism-related gene mRNA level of the hepatopancreas in grass carp. Different lowercase letters in the figure indicate significant differences. p_1, the effect of oil levels on data; p_2, the effect of oil sources on data; p_3, effect of the interaction between oil levels and oil sources on the data. $p < 0.05$ means significant difference. ($n = 6$).

The mRNA relative expression of lipolysis-related genes *ppar-α* and *ucp-1* in the PASO group was higher than that in the SO group, while the HPASO group showed lower relative expression than the HSO group, although the differences were not significant ($p > 0.05$). The mRNA expression level of *cpt-1* in the PASO group showed a significant increase ($p < 0.05$). Two-way ANOVA analysis showed that both oil levels and oil sources had significant and interactive effects on *cpt-1* ($p < 0.05$).

4. Discussion

The present results indicate that SR, SGR, and FCR were not affected by the high lipid levels of the diets, which is consistent with previous research [37]. They were also not significantly affected by PASO, which may be related to the proximity of the gross energy of the four dietary groups on one hand, and on the other hand, it may be due to satiation feeding, where fish may have adjusted their energy intake to meet other needs [38].

Therefore, this suggests that the addition of PASO to diets of normal or high lipid levels is beneficial for the growth of grass carp.

The present VSI values were significantly higher in the high-lipid-level diets of the HSO and HPASO groups than those in the SO and PASO groups (Table 4), which was similar to the previous results in turbot: higher VSI values of fish were found in the higher-lipid diet [39], showing the higher lipid deposition was caused by a high-lipid diet. Similarly, in the present study, higher lipid accumulation and greater accumulation of lipid droplets was found in the hepatopancreas of grass carp in the HSO and HPASO groups than in the SO and PASO groups (Table 5 and Figure 1), which was in agreement with previous results that high-fat diets trigger lipid accumulation [40,41]. Immoderately high lipid in the diet usually results in the excessive accumulation of lipid in the tissue of fish and probably caused fatty liver disease [42,43] and these results have also been previously reported in blunt snout bream [41], common carp [40,44], tilapia [45], and grass carp [46,47]. Although the present addition of PASO in normal- or high-lipid diets showed no significant effect on the lipid accumulation in the hepatopancreas of grass carp, the PASO group showed significantly reduced adipocyte size under a two-way ANOVA analysis (Figure 2b). As LNA was previously reported to reduce lipid accumulation in fish [17,48,49], and PASO is rich in LNA, this was postulated to cause the reduction of lipid accumulation in the adipocyte of the PASO group in the present study.

The synthesis and decomposition of lipid were regulated by a series of key enzymes and transcription factors [50]. The *srebp-1c* gene directly regulates the expression of *fas*, a key gene in the de novo biogenesis of fatty acids [51,52]. In addition, *ppar-α* and *cpt-1* regulate key enzymes in the β-oxidation pathway to facilitate the process [53]. In the present study, PASO decreased the relative expression of *ppar-γ*, *cebp-α*, and *srebp-1c*, i.e., lipogenesis-related genes, and it simultaneously increased the relative expression of *ppar-α* and *cpt-1*, i.e., lipolysis-related genes. Previous result also showed that the high content of LNA regulated the lipid metabolism by affecting the expression of genes related to lipid synthesis and catabolism [54,55] and that LNA decreased the expression of *srebp-1c* in grass carp [17] and increased the expression of *cpt-1* in oriental river prawn (*Macrobrachium nipponense*) [56] as well as *ppar-α* in goat [57]. In the present study, the rich LNA in PASO was postulated to inhibit the lipid accumulation in grass carp by inhibiting the de novo synthesis and promoting β-oxidation of fatty acids.

In our results, the fatty acid composition showed a significant correlation between that in the fish and in the diets (Tables 6–8), suggesting that the fatty acid composition of the fish was significantly affected by the dietary fatty acid composition, which is in line with previous results in fish [58–60]. LA and LNA are the precursors of n-6 and n-3 PUFA, respectively, and their conversion relies on the processes of chain elongation and desaturation [36,61]. Previous studies have confirmed the desaturase function of the *fads* and *elvols* for LA and LNA in grass carp with the ability to biosynthesize LC-PUFAs [62,63]. In the present study, the significant decrease in the LNA content and the significant increase in the n-3 PUFA content in the muscle of fish compared to the diet suggests that the LNA in the PASO diet may be converted to LC-PUFAs in grass carp muscle. On the other hand, there was no significant change in LC-PUFA in the hepatopancreas and abdominal fat compared to the diet, and presumably, LNA is used for oxidative energy supply in these two tissues. Therefore, the grass carp in this study had the ability to biosynthesize LNA into LC-PUFA.

5. Conclusions

In conclusion, in the present study, high-fat diets and PASO did not significantly affect the growth of grass carp, while PASO reduced the lipid accumulation of grass carp fed diets with normal lipid levels. The fatty acid composition of the grass carp was significantly affected by their diets, and grass carp had the ability to biosynthesize LNA into LC-PUFA.

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investigation; M.Z., investigation; J.Z., methodology, supervision, funding acquisition, project administration, and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All experimental procedures conducted in this study were performed following the guidelines and regulations set by Northwest A&F University of Animal Experimentation Ethics Committee, Yangling, China (No. DK2022007). This study followed the guidelines provided by the National Council for Control of Animal Experimentation (CONCEA) for the care and use of laboratory animals.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

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