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Research Article

Effects of Dietary Lipid Levels on the Lipid Deposition and Metabolism of Subadult Triploid Rainbow Trout (*Oncorhynchus mykiss*)

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A growth trial was conducted to evaluate the effects of dietary lipid levels on the lipid deposition and metabolism of subadult triploid rainbow trout. Diets with low (148 g lipid kg^{-1} diet), moderate (228 g lipid kg^{-1} diet), and high (294 g lipid kg^{-1} diet) crude lipid contents were fed to quadruplicated groups of fish (233 g ± 0.2 g) for 80 days, and they were named as LL, ML, and HL, respectively. Results showed that the lowest and highest values of condition factor and hepatosomatic index were shown in the LL group, respectively, while the HL group obtained the highest liver redness value, plasma total cholesterol, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol contents ($P < 0.05$). The viscerosomatic index and plasm glucose, nonesterified fatty acids, and triglyceride contents were comparable among groups ($P > 0.05$). As for lipid deposition, viscera and muscle were the main lipid storage place for triploid rainbow trout when tissues' weight is taken into consideration. Overall quantitative PCR showed that the lipid uptake, triglyceride, and fatty acid catabolism were upregulated, but glycolysis was downregulated as dietary lipid increased. The expression of genes involved in lipogenesis, lipoprotein clearance, very low-density lipoprotein assembly, and glycogenesis was not affected by dietary lipid levels ($P > 0.05$). In summary, subadult triploid rainbow trout store lipid mainly in viscera and muscle; it could maintain hepatic lipid homeostasis when fed with dietary lipid levels ranging from 150 to 300 g kg^{-1} by regulating lipid uptake and switching energy supply between glycolysis and fatty acids β-oxidation.

1. Introduction

Feed is a critical factor in intensive aquaculture, and dietary lipids are the predominant source of energy for fish. To save dietary protein as an energy source and increase feed efficiency [1], high lipid diets (HLD) are widely applied in aquaculture nowadays. However, numerous studies have revealed that a sustained high lipid intake induces ectopic lipid accumulation and compromises the growth and health of farmed fish, suggesting impaired lipid homeostasis [2]. Thus, understanding how to maintain lipid homeostasis is necessary for farmed fish.

Abnormal fluctuations of endogenous lipid and its derivatives can cause metabolic syndromes in most vertebrates [3], and animals have developed an accurate and complicated metabolic system to adapt to different nutritional states. The liver plays a central role in maintaining metabolic homeostasis by coordinating gene expression programs in response to dietary and systemic signals [4, 5]. Studies in some fishes have shown that accelerating lipid biosynthesis to satisfy the physiological lipid requirement is necessary when dietary lipid is very limited; in contrast, dietary lipid toward an upper limit often leads to unwanted deposition in the liver (or other tissues), inducing a condition referred to as fatty liver disease [6–8]. Compared with most farmed fishes with lipid requirements below or around 100 g kg^{-1} diet, salmonids could tolerate high lipid diets well [9]. According to our previous study, feeding triploid rainbow

trout diets with dietary lipid levels ranging from 228 to 294 g kg^{-1} diet showed no differences in growth performance and health [10]. Therefore, triploid rainbow trout could act as a good model to study the adaptive strategies. Based on the molecular biology applied to fish, several lipometabolic genes have been cloned, and preliminary functions have also been illustrated [4, 11–13]. However, no systematic gene expression is analyzed in triploid rainbow trout fed with diets of various lipid levels.

To avoid problems caused by early sexual maturation, sterile triploid rainbow trout has been investigated for a long time [14, 15]. Besides, the application of triploids in aquaculture can also reduce gene flow and invasive species risks [16]. Therefore, the fast growth of triploid rainbow trout farming has shown in China which is not the original home of this fish [17]. Understanding the physiology and its response to nutrients is still limited for this fish.

Thus, the objectives of the present study were to investigate how triploid rainbow trout maintain lipid homeostasis by examining the response of key metabolic genes.

2. Materials and Methods

2.1. Fish, Feed, Feeding, and Sampling. The present study was performed in accordance with the Standard Operation Procedures of the Guide for the Use of Experimental Animals of Qinghai University. Female triploid rainbow trout was obtained from a local fish farm (Longyangxia, Qinghai, China); a total of 1200 healthy fish with an initial weight of 233 ± 0.2 g from the same group were evenly divided into 12 freshwater net cages (3 × 3 × 6 m) and were fed with a commercial diet for two weeks to adapt to the environment. Three isonitrogenous diets (dietary protein level: 460 g kg^{-1}) with graded lipid levels of 148, 228, and 294 g kg^{-1} (named as LL, ML, and HL, respectively) were formulated and stored as previously specifically formulated, prepared, and stored as previously reported [18]. The formulation and proximate compositions of the experimental diets are shown in Table 1. After the adaption, fish from each cage was randomly fed with one of the three experimental diets for 80 days in Longyangxia reservoir. There were three experimental treatments with four replicates (net cages) each. The water temperature remained at 8-16°C, and dissolved oxygen was higher than 7 mg l^{-1}. After the feeding trial, all fish in each cage were counted and weighed; the final weight of fish from the HL group reached an average value of 682 g, which was significantly higher than that of the LL group (610 g) but not significantly different from the ML group (655 g). Besides, the feed conversion ratio of ML (0.98) and HL (0.97) groups was significantly higher than that in the LL group (1.07) [10].

Body weight and length, viscera, and liver weight of three fish per net cage were measured to calculate condition factor (CF), viscerosomatic index (VSI), and hepatosomatic index (HSI). The liver redness value was measured according to Ma et al. [17]. Blood samples of three randomly selected fish per net cage were taken from the caudal vein, collected in heparinized syringes, and centrifuged (4000 × g, 10 min) to separate plasma. Samples of liver tissue from the bled fish were subsequently dissected. Then, samples of plasma and liver tissue of three fish with similar volume or size from each net cage were pooled as one biology replicate and stored in liquid nitrogen for further analysis. For histological analysis, liver samples with 1 cm^3 were frozen in liquid nitrogen and stored at -20°C for making frozen sections.

2.2. Chemical and Histological Analysis. The experimental diets were analyzed in triplicated the same as previously described [18]. Plasma glucose, triglyceride, total cholesterol, higher-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assayed the same as described in Meng et al. [10]. Plasma nonesterified fatty acids (NEFA) were measured using a Cu-NEFA coextraction-based colorimetric assay kit (Nanjing Jiancheng Bioengineering Inc., Nanjing, China) according to Falholt et al. ([19]). Lipid content of whole fish, visceral, liver, and muscle samples taken from posterior fillet were extracted using chloroform : methanol (2 : 1, v/v) according to the method of Ma ([17]).

For hepatic histological analysis, one sample in each cage was randomly chosen, and they were sliced into 7 μm thick sections by using a freezing microtome and stained with oil red.

2.3. Real-Time Quantitative PCR (qRT-PCR) Analysis of Genes. Total RNA was extracted from the liver of triploid rainbow trout (four biological replicates for each treatment) using RNA simple total RNA kit (TIANGEN, China). RNA quality and quantity were assessed using agarose gel (1%) electrophoresis and spectrophotometric (A260:280 nm ratio) analysis, respectively. RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent Kit (TaKaRa, China).

Specific primer of the genes was designed referring to the relevant cDNA sequences of rainbow trout (Table 2). qRT-PCR was conducted with the Multiwell Plate 96 reaction system (LightCycler® 480, Roche, USA) in a final volume of 20 μl containing 0.5 μl (10 μM) each of forward and reverse primer, 10 μl SYBR® Premix Ex Taq™ II (2x), 1 μl cDNA, and 8 μl ddH_2O. The relative abundance of genes was normalized by the expression of β-actin and calculated using the $2^{-\Delta\Delta Ct}$ method.

2.4. Calculations.

$$\text{Condition factor (CF)} = 100 \times \frac{[\text{body weight (g)}]}{[\text{body length (cm)}]^3},$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times \frac{[\text{viscera weight (g)}]}{[\text{body weight (g)}]},$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times \frac{[\text{hepatic weight (g)}]}{[\text{body weight (g)}]}. \quad (1)$$

2.5. Statistical Analysis. Data were assessed by one-way ANOVA using SPSS 19.0 for Windows. When differences were significant ($P < 0.05$), Tukey's test was used to compare the means among individual treatments.

Table 1: Formulation and proximate compositions of the experimental diets (g kg^{-1}).

Ingredients	LL diet (148 g kg^{-1})	ML diet (228 g kg^{-1})	HL diet (294 g kg^{-1})
Fish meal[1]	600	600	600
Wheat meal[1]	120	120	120
Corn starch[1]	142.2	62.2	2.2
Fish oil	80	160	220
Soybean oil	30	30	30
Mineral premix[2]	5	5	5
Vitamin premix[3]	5	5	5
Ca $(H_2PO_4)_2$	8	8	8
Choline chloride	3	3	3
Mold inhibitor	1	1	1
Antioxidants	0.5	0.5	0.5
Betaine	5	5	5
Astaxanthin[4]	0.3	0.3	0.3
Total	1000	1000	1000
Proximate analysis ($n = 3$)			
Moisture (g kg^{-1} diet)	37	43	42
Crude protein (g kg^{-1} diet, dry basis)	461	458	455
Crude lipid (g kg^{-1} diet, dry basis)	148	228	294
Ash (g kg^{-1} diet, dry basis)	111	109	109
Gross energy (kJ g^{-1} diet)	21.1	22.9	24.2

[1]Fish meal; crude protein, 695 g kg^{-1}; crude lipid, 80 g kg^{-1}. Wheat meal; crude protein, 150 g kg^{-1}; crude lipid, 12 g kg^{-1}. Corn starch; crude protein, 3 g kg^{-1}; crude lipid, 2 g kg^{-1}. [2]Mineral premix included the following (mg kg^{-1} diet): sodium, 1500; iron, 3000; copper, 90; zinc, 1500; manganese, 800; selenium, 4.3; iodine, 21; and cobalt, 3. [3]Vitamin premix included the following (each kg^{-1} diet): vitamin A, 50 KIU; vitamin D3, 20 KIU; vitamin E, 390 mg; vitamin K, 150 mg; vitamin B1, 120 mg; vitamin B2, 165 mg; vitamin B6, 130 mg; vitamin B12, 0.5 mg; biotin, 2.4 mg; folic acid, 75 mg; inositol, 1200 mg; niacin, 670 mg; and ascorbic acid, 2500 mg. [4]Astaxanthin: 10% (CAROPHYLL®, DSM, Netherlands).

3. Results

3.1. Organ Indexes and Plasma Biochemistry. As shown in Table 3, fish from the ML and HL groups had significantly higher values of CF and a lower value of HSI than the LL group ($P < 0.05$). No significant difference was found for VSI ($P > 0.05$). As the increasing level of dietary lipid, liver redness value gradually increased with a significantly higher value observed in HL treatment than that in the LL group ($P < 0.05$).

No significant difference was found in plasma glucose, NEFA, and triglyceride ($P > 0.05$). As dietary lipid levels increased, plasma total cholesterol, HDL-C, and LDL-C contents increased and reached their maximum average value in the HL group ($P < 0.05$).

3.2. Lipid Deposition. The lipid content of whole fish as well as viscera, liver, and muscle tissues is shown in Table 4. The significantly higher value of whole fish and visceral lipid content (calculated as g 100 g tissue wet weight^{-1}) was observed in the ML and HL groups ($P < 0.05$). As dietary lipid content increased, the lipid content of the liver and muscle gradually increased and reached their maximum average value in the HL group ($P < 0.05$). When the weight of tissues and the whole body was considered (calculated as g tissue of a 100 g fish^{-1}), the ML and HL groups obtained a significantly higher value of visceral lipid content ($P < 0.05$); no significant difference in hepatic lipid content was observed ($P > 0.05$); the significant highest value of muscle lipid content was found in the HL group ($P < 0.05$).

3.3. The Histological Appearance of the Liver. Results of the histological appearance of the liver are shown in Figure 1. By applying oil red staining, lipid droplet was stained with red color. The lipid droplet did not obviously alter the regular shape and size of hepatic cells in general. Compared with liver tissue of the LL group, the ML and HL groups obtained more intensive lipid droplets; the hepatic histological appearance in the ML and HL groups seems not obviously different.

3.4. The Relative mRNA Expression Levels of Hepatic Metabolism Genes. Data on hepatic mRNA expression of genes related to lipid uptake are shown in Figure 2. Dietary lipid levels did not significantly influence the gene expression of CD36 and FATP ($P > 0.05$). The significantly higher expression levels of LPL, FABP3, and FABP10 genes were observed in the ML and LL groups ($P < 0.05$). The significantly highest gene expression level of FABP was shown in the HL group ($P < 0.05$).

Data on hepatic mRNA expression of genes related to triacylglycerol synthesis and catabolism are shown in Figure 3; dietary lipid levels did not significantly influence the gene expression of hepatic triacylglycerol and fatty acid synthesis (ACCα, FAS, ME, G6PDH, and DGAT2) ($P > 0.05$). For

Table 2: Primer sequences for real-time quantitative PCR.

Gene symbol	Gene name	Primer sequence (5′-3′)		GenBank accession no.
Reference gene				
β-Actin	β-Actin	F: TACAACGAGCTGAGGGTGGC	R: GGCAGGGGTGTTGAAGGTCT	AJ438158.1
Lipid uptake				
LPL	Lipoprotein lipase	F: GGACGTTGGGGAGCTGCTTA	R: ATTGAGTCTCCCCGGCCTTG	NM_001124604.1
CD36	Cluster of differentiation 36	F: GTCGTGGCTCAAGTCTTCCA	R: TCAAATACTCGGCTCGCCTC	AY606034.1
FATP	Fatty acid transport protein	F: TGCTGGTGGGAAGGATCAAC	R: TCCCCTGACAGATATGCCGA	XM_021558559.1
FABP	Fatty acid binding protein	F: ACGGCAAGGAGACCAAGTTT	R: TACGCACTGCCACAATGTCT	JN413683.1
FABP3	Fatty acid binding protein 3	F: ATGGCTGAGGCATTCG	R: CATCTTACCACCGTCTATC	NM_001124713.1
FABP10	Fatty acid binding protein 10	F: CTTCGTCATCACCTCCAA	R: CCACTGTCAGCGTCTCA	XM_021595306.1
Triacylglycerol synthesis and catabolism				
ACCα	Acetyl-CoA carboxylase α	F: CCCATTCGCCTTTTCCTTAC	R: ACCCTGCGTGTCCCCATA	XM_021623129.1
FAS	Fatty acid synthase	F: TCTAGAGACGCCACCTTCGA	R: TGCAGTTTCTCCTCAGCCAG	XM_021581290.1
ME	Malic enzyme	F: CCATTGGAGGGGCGTTTACT	R: GGGGTTACTCAGGGCGAAAA	XM_021597955.1
G6PDH	Glucose-6-phosphate dehydrogenase	F: ACCACTTGCTCCAGATGCTC	R: TACTGCCCCAACACCACATC	XM_021615620.1
DGAT2	Diacylglycerol O-acyltransferase 2	F: TGCCCAGTGAACCGTAACTC	R: CCCTTGCGGTTATTCAGGGT	XM_021623520.1
ATGL	Adipose triglyceride lipase	F: GCTGCAGAGTGTTCTTTCGC	R: GGGAGTGTTGCAATTCCAGC	KM980090.1
HSL1	Hormone-sensitive lipase subtype 1	F: CGACCTACTGCGTGGACTAC	R: TGGCCCATATTCCGCAGTTT	NM_001197209.1
CPT1	Carnitine palmitoyl transferase 1	F: TACAGCTGGCCCAATTCAGG	R: TCGCAGTGTTCTTGTCCTCC	AF327058.3
ACO	Acetyl-CoA oxidase	F: TTGGGCCTCATCATTGCAGT	R: ACTGGGTCTGGTGCTCAATG	XM_021613038.1
ACCβ	Acetyl-CoA carboxylase	F: CAGCTTGGTAAACAGCCTGG	R: TCCGCACATTCTTCCAGTCC	XM_021620985.1
VLDL assembly and lipoprotein clearance				
ApoB	Apolipoprotein B	F: TCCAGCCATCAACCTCCCTA	R: TCGCCATAGAGCTTGCCAAA	XM_021585085.1
MTP	Microsomal triglyceride transfer protein	F: ACGTGACTGTGGACATGGAC	R: ATCTGCATGCACACCAGGAA	XM_014195517.1
HL	Hormone-sensitive lipase	F: CGACCTACTGCGTGGACTAC	R: TGGCCCATATTCCGCAGTTT	NM_001197209.1
LDLR	Low-density lipoprotein receptor	F: GTGACAGGCAGGGACATCAA	R: TGGCTGTTTGAGGTTGTGGT	XM_021558198.1
LRP-1	Lipoprotein receptor-related protein 1 like	F: GCACCAGAGCCTGTATGTGT	R: AGAGCAGGAAGGAGCCCATA	XM_021565892.1
SRBI	Scavenger receptor class BI	F: TGGAGCAGAGAGGACCCTAC	R: GTGACCACATCCGACTCGTT	XM_021590607.1
Transcription factors				
PPARα	Peroxisome proliferator-activated receptor α	F: CACTCCACCCTTCGTCATCC	R: CCTCAGCCTCCTTCTCAAGC	HM536190.1
PPARβ	Peroxisome proliferator-activated receptor β	F: GGGGTACACGTGTTCTACCG	R: GTACTTCAGCAGCGTCACCT	HM536191.1
PPARγ	Peroxisome proliferator-activated receptor γ	F: GAGCTGGACATGAACGACCA	R: TGTGCCGTCCTTGTTCATGA	NM_001197212.1
SREBP1	Sterol regulatory element-binding protein 1	F: TCCTCTCCCTCAATCCCCTG	R: CGAGTCAGCTGCGTTGTCT	KP342261.1
LXR	Liver X receptor	F: CCAGAGTCAGGACCCTCAGA	R: CTCCTGGACCGACATGATGG	FJ470291.1

Table 2: Continued.

Gene symbol	Gene name	Primer sequence (5′-3′)		GenBank accession no.
Carbohydrate metabolism				
GK	Glucokinase	F: AGATCACTGTGGGCATCGAC	R: GATGTCACAGTGAGGCGTCA	AF053331.2
PK	Pyruvate kinase	F: GTTCCCTGTCGAGTCTGTGG	R: CAGACGACGAAGCTCCTCAA	XM_021622264.1
G6Pase	Glucose-6-phosphatase	F: GCTGACCTGCATACCACCTT	R: CAGCCACCCAGATGAGCTTT	XM_021575943.1
FBPase	Fructose 1,6-bisphosphatase	F: CCACTGGATGGATCTTCCAACA	R: CCCTCTCATTGGGCTCATCG	AF333188.1
PEPCK	Phosphoenol pyruvate carboxykinase	F: CGGTGTGTTTGTAGGAGCCT	R: ACGTGGAAGATCTTGGGCAG	NM_001124275.1

Table 3: Organ indexes and plasma biochemical parameters of triploid rainbow trout fed the experimental diets for 80 days[1].

	LL	ML	HL	Pooled S.E.M.[2]	F value	Pr > F
Organ indexes						
Condition factor[3]	1.86[a]	2.01[b]	2.00[b]	0.02	6.106	0.004
Viscerosomatic index (%)[4]	13.19	13.81	14.23	0.25	1.645	0.211
Hepatosomatic index (%)[5]	1.79[b]	1.39[a]	1.27[a]	0.05	17.744	0.000
Liver redness value[6]	26.00[a]	26.92[ab]	29.25[b]	0.45	6.071	0.006
Plasma biochemistry parameters						
Glucose (mmol l^{-1})	4.2	4.3	4.2	0.13	0.044	0.957
NEFA (umol l^{-1})[7]	1258	1610	1385	124	0.557	0.596
Triglyceride (mmol l^{-1})	4.79	4.57	4.44	0.12	0.605	0.572
Total cholesterol (mg dl^{-1})	9.52[a]	10.68[ab]	11.09[b]	0.27	5.545	0.027
HDL-C (mmol l^{-1})[8]	3.96[a]	4.36[ab]	4.51[b]	0.10	5.471	0.032
LDL-C (mmol l^{-1})[9]	2.35[a]	2.97[ab]	3.22[b]	0.15	6.209	0.020

[1]Values are means of four replicates. Values in a row that do not have the same superscript are significantly different at $P < 0.05$ based on Tukey's multiple range test. [2]Pooled S.E.M.: pooled standard error of means. [3]Condition factor (CF) = 100 × [body weight (g)]/[body length (cm)]^3. [4]Viscerosomatic index (VSI, %) = 100 × [viscera weight (g)]/[body weight (g)]. [5]Hepatosomatic index (HSI, %) = 100 × [hepatic weight (g)]/[body weight (g)]. [6]Liver redness value was assessed visually by using the color card (SalmoFan™, DSM, Netherlands). [7]NEFA: nonesterified fatty acid. [8]HDL-C: high-density lipoprotein-cholesterol. [9]LDL-C: low-density lipoprotein-cholesterol.

Table 4: Lipid content of whole fish, viscera, liver, and muscle of triploid rainbow trout fed the experimental diets for 80 days[1].

	LL	ML	HL	Pooled S.E.M.[2]	F value	Pr > F
Whole fish						
(g/100 g wet weight)	21.56[a]	24.33[b]	26.66[b]	0.64	12.386	0.001
Visceral						
(g/100 g tissue wet weight)	37.69[a]	44.05[b]	45.07[b]	1.17	6.419	0.008
(g/tissue of a 100 g fish)	4.97[a]	6.08[b]	6.42[b]	0.19	13.308	0.000
Liver						
(g/100 g tissue wet weight)	7.85[a]	9.21[ab]	9.94[b]	0.35	5.801	0.024
(g/tissue of a 100 g fish)	0.144	0.139	0.135	0.004	1.210	0.343
Muscle						
(g/100 g tissue wet weight)	8.75[a]	9.10[ab]	10.57[b]	0.31	4.049	0.030
(g/tissue of a 100 g fish)	5.64[a]	5.81[a]	6.74[b]	0.17	5.568	0.010

[1]Values are means ($n = 4$). Values in a row that do not have the same superscript are significantly different at $P < 0.05$ based on Tukey's multiple range test. [2]Pooled S.E.M.: pooled standard error of means.

catabolism, the lowest gene expression level of HSL1 and CPT1 was shown in the LL group ($P < 0.05$); the ML and HL groups were not significantly different ($P > 0.05$). The gene expression level of ACO was higher in the HL group than the LL and ML groups ($P < 0.05$). Gene expression levels of ATGL and ACCβ were not affected by dietary lipid levels ($P > 0.05$).

Data on hepatic mRNA expression of genes related to very low-density lipoprotein (VLDL) assembly and lipoprotein clearance are shown in Figure 4. Dietary lipid levels did not affect the hepatic gene expression of VLDL assembly (APOB and MTP) and lipoprotein clearance (HL, LDLR, LRP-1, and SRBI) ($P > 0.05$).

Data on hepatic mRNA expression of genes related to transcription factors are shown in Figure 5. No significant difference was found in the expression of PPARβ, SREBP1, and LXR genes ($P > 0.05$). The significantly highest expression of PPARα was shown in the HL group ($P < 0.05$). Besides, the HL group also obtained a significantly higher PPARγ gene expression level than that of the LL group ($P < 0.05$).

Data on hepatic mRNA expression of genes related to carbohydrate metabolism is shown in Figure 6. No significant difference was found in hepatic gluconeogenesis (G6Pase, FBPase, and PEPCK) ($P > 0.05$). For glycolysis, the gene expression of GK decreased markedly as dietary lipid level increased ($P < 0.05$). The significantly lowest gene expression of PK was found in the HL group ($P < 0.05$).

4. Discussion

Studies have shown that the lipid deposition pattern varies markedly in different fish species [20]. It is generally accepted that fish accumulate excess lipid nonspecifically in

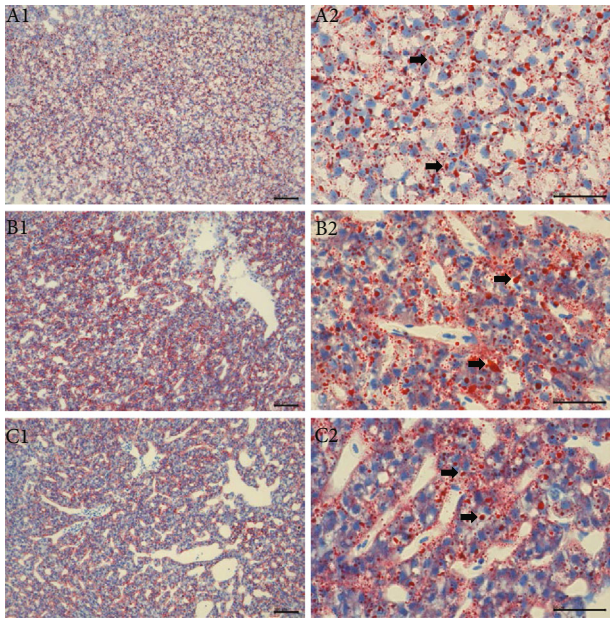

Figure 1: Histological appearance of the liver from triploid rainbow trout fed the LL (a1, a2), ML (b1, b2), and HL (c1, c2) diet, respectively. (a1, b1, c1) Magnification ×100, bar = 100 μm. (a2, b2, c2) Magnification ×400, bar = 50 μm. The arrow points to the fat droplets accumulated in liver cells.

organs such as the liver and muscle, as well as in specifically dedicated adipose tissues [21]. Studies comparing diploid and triploid rainbow trout revealed that lipid deposition and metabolism are quite different ([22–24]; Wang et al., 2017). However, the lipid deposition pattern of triploid rainbow trout feeding with various dietary lipid levels is still unknown. Therefore, it is necessary to evaluate the triploid rainbow trout separately from their diploid counterparts.

Results showed that fish fed with ML and HL diets obtained significantly higher values of CF. This result is further supported by the elevated lipid content of whole fish. Generally, the increase in dietary lipid levels is usually associated with an increased lipid deposit in the viscera cavity [25, 26]. Although there is no significant difference for VSI in the present study, the lipid content of visceral was higher in the ML and HL groups compared with the LL group. Similar results were also observed in a study on rainbow trout in which the fat content in the visceral and whole body was higher in fish fed with 275 g kg^{-1} than those fed with 126 g kg^{-1} dietary lipid content [25]. Unexpectedly, the reduced HSI value was found in the ML and HL groups. By searching literatures, we found that decreased HSI was also observed both in rainbow trout [27, 28], sea bass [29], and grass carp [6] feeding with high dietary lipid levels. Some attribute the decreased HSI to low hepatic glycogen content caused by low dietary starch [29]. A study that investigated the effects of essential fatty acids (EFA) on rainbow trout found similar reduced HSI when fish fed with excess EFA [30]. Besides, long-chain PUFA from dietary fish oil was also found to prevent the growth of adipocyte size and adipose tissue mass in rats [31]. Therefore, it is possible that the low starch and the high amount of fish oil supplement in the feed of ML and HL diets could reduce HSI.

Although the relatively increased lipid content of the liver in the HL group (9.94 g/100 g tissue wet weight) was observed, due to the reduced HSI, the liver may not be the main lipid deposition place for triploid rainbow trout since the lipid deposition in liver only constitutes about 0.14 g/tissue of a 100 g fish. Although the quantitative lipid content of muscle (average value ranged from 8.75 to 10.57 g/100 g tissue wet weight) is less than that for visceral (average value ranged from 37.69 to 45.07 g/100 g tissue wet weight), due to the large proportion, the muscle tissue (average value ranged from 5.64 to 6.74 g/tissue of a 100 g fish) contributes equally as visceral tissue (average value ranged from 4.97 to 6.42 g/tissue of a 100 g fish) to the lipid deposition from the whole fish aspect. This is different from diploid counterparts. A study in diploid rainbow trout of a similar size observed the existence of a fattening effect of fat-enriched diets on the liver, viscera, and muscle; however, the increase in viscera and muscle lipid contents was responsible for 63% and 37% of the increase of whole-body lipid contents between the T15 and T30 diets [32]. Therefore, the lipid metabolism of triploid rainbow trout could be different.

The liver plays an important role in balancing the lipid homeostasis of fish. The reduced HSI and higher liver redness value, as well as the hepatic histology, also suggested that rainbow trout could maintain lipid homeostasis without getting fatty liver disease. This could be further supported by the comparable level of plasma glucose, NEFA, and triglyceride among different treatments. To understand how fish liver maintains lipid homeostasis, gene expressions were analyzed. Absorbed by the intestine, the exogenous lipids enter the blood circulation in the form of chylomicron (CM). During transport, LPL on the inner wall of capillaries breaks down triglyceride (TAG) into NEFA for tissue use. Then, FA uptake is by FA transporters such as CD36, FATP, and FABPs. Both LPL and FABPs played an intermediary role in lipid homeostasis and are regulated according to the nutritional state [4]. The present results showed that low lipid feed decreased the FA uptake as reflected by the decreased gene expression of LPL. Similar results were also shown in a study with rainbow trout, in which fish fed feed with a dietary lipid level of 150 g kg^{-1} obtained a lower value of LPL mRNA expression than that with 210 g kg^{-1} dietary lipid level [33]. Lu et al. [8] and Yuan et al. [34] also reported that a high-lipid diet significantly upregulated LPL in the liver of blunt snout bream and grass cap.

FABPs play a vital role in lipid uptake, transport, and overall lipid homeostasis; several FABP genes were identified in rainbow trout [35], and these transporters in fish may have the similar roles as in mammals [13, 36, 37]. The present results indicated that genera and high lipid feed could enhance the β-oxidation of FA (reflected by gene expression of FABP3 and FABP10); besides, in accordance with the elevated hepatic lipid content, fish fed with high lipid feed result in hepatic lipid deposition (reflected by gene expression of FABP11). The complexity of FABPs making results of FABPs is quite controversial. For example, a different result was observed in a research on diploid rainbow trout,

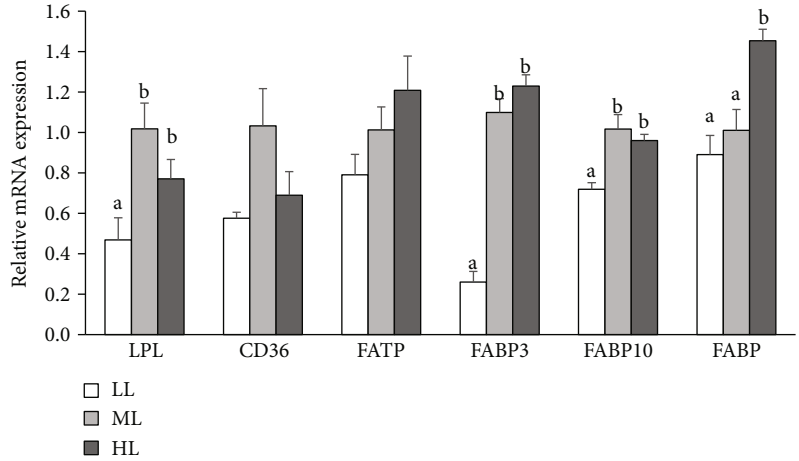

Figure 2: The mRNA expression of genes related to lipid uptake in the liver of triploid rainbow trout fed the experimental diets for 80 days. Values are expressed as means ± S.E.M. ($n = 4$). Statistical significance was evaluated using one-way ANOVA, followed by Tukey's test. Values with different letters on columns statistically differ at $P < 0.05$.

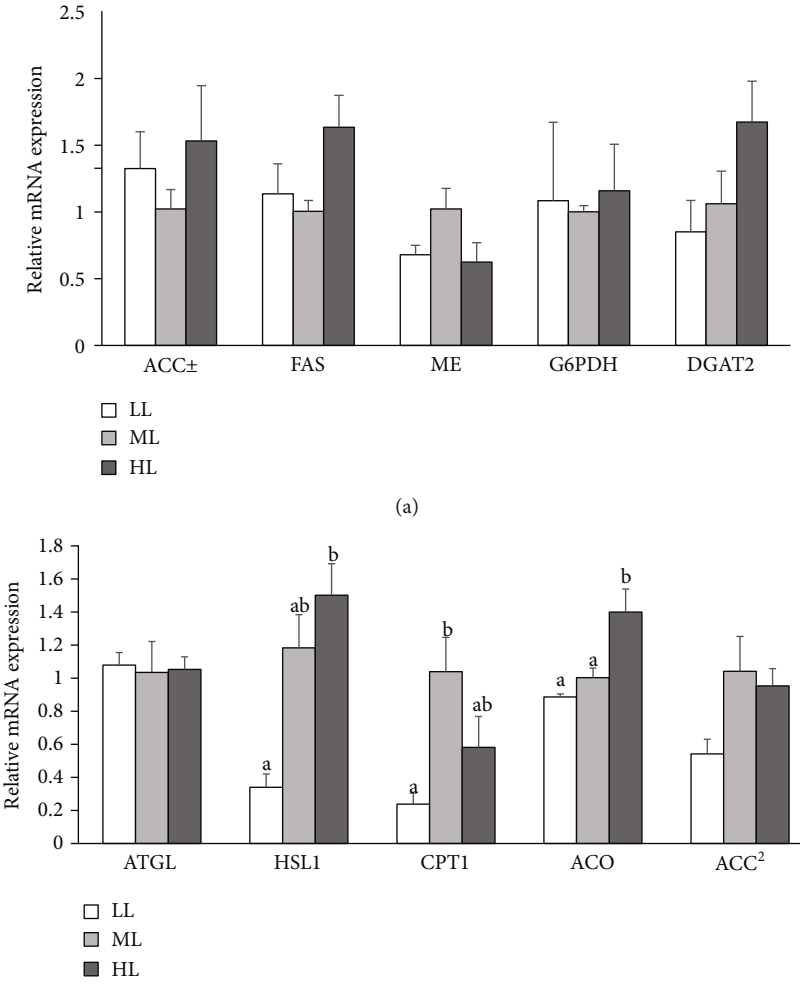

Figure 3: The mRNA expression of genes related to triacylglycerol synthesis (a) and catabolism (b) in the liver of triploid rainbow trout fed the experimental diets for 80 days. Values are expressed as means ± S.E.M. ($n = 4$). Statistical significance was evaluated using one-way ANOVA, followed by Tukey's test. Values with different letters on columns statistically differ at $P < 0.05$.

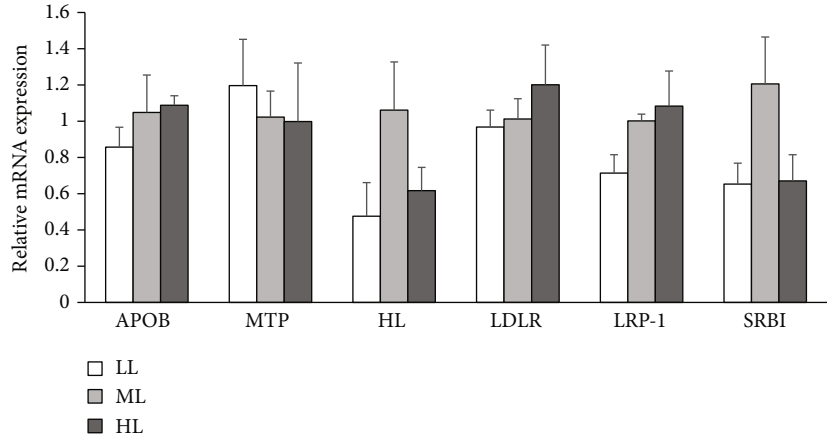

Figure 4: The mRNA expression of genes related to VLDL assembly (APOB and MTP) and lipoprotein clearance (HL, LDLR, LRP-1, and SRBI) in the liver of triploid rainbow trout fed the experimental diets for 80 days. Values are expressed as means ± S.E.M. ($n = 4$). Statistical significance was evaluated using one-way ANOVA, followed by Tukey's test. Values with different letters on columns statistically differ at $P < 0.05$.

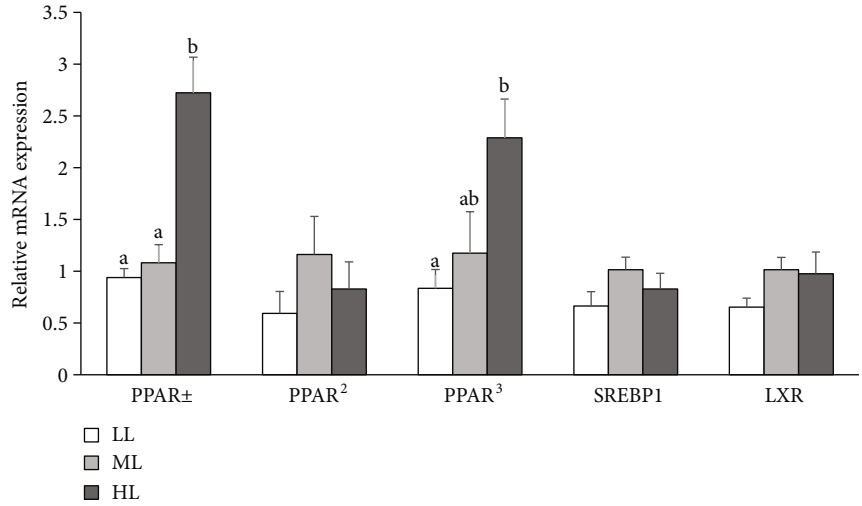

Figure 5: The mRNA expression of PPARs, SREBP1, and LXR in the liver of triploid rainbow trout fed the experimental diets for 80 days. Values are expressed as means ± S.E.M. ($n = 4$). Statistical significance was evaluated using one-way ANOVA, followed by Tukey's test. Values with different letters on columns statistically differ at $P < 0.05$.

in which fish fed with 250 g kg^{-1} dietary lipid levels could significantly decrease the value of FABP compared with fish fed with 100 g kg^{-1} dietary lipid [38]. These potential roles must be confirmed in further studies.

Under normal physiological conditions, there is a balance between the uptake and secretion of lipid in the liver [39]. The present results found that although the endogenous lipoprotein uptake was consistent (revealed by gene expression of LDLR, LRP-1, HL, and SRBI), the elevated exogenous lipid uptake (revealed by gene expression of LPL and FABPs) increased the total lipid uptake in fish from the ML and HL groups. Excess TAG secretion is transported in the form of VLDL to the peripheral tissues for storage or utilization. Therefore, the VLDL assembly and secretion are crucial for lipid homeostasis. It has been suggested that a low level of lipid transports out of the liver, alterations of lipid transport, and elevated lipid uptake contributed to the fatty liver of fish fed a high-lipid diet [8, 40]. However, it was strange that the synthesis of VLDL was not affected by the level of dietary lipid (APOB and MTP) suggesting a consistent hepatic lipoprotein output of triploid rainbow trout when fed with different dietary lipid levels. This is in accordance with the consistent level of plasma TG and NEFA. However, the elevated plasma total cholesterol, HDL-C, and LDL-C suggested the complexity between mRNA expression and physiological indicators. Cholesterol metabolism deserves further studies.

TAG and fatty acid metabolism are also pivotal factors affecting lipid homeostasis. Consistent gene expression of ACCα, FAS, ME, G6PDH, and DGAT2 confirmed that the

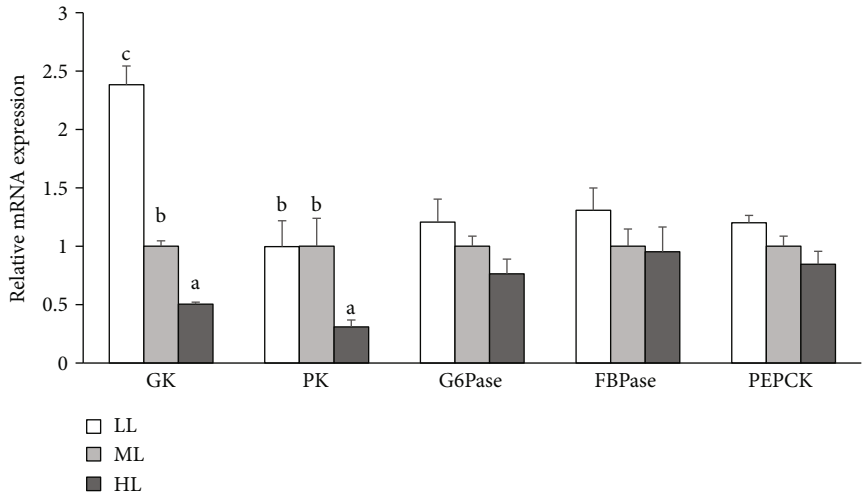

Figure 6: The mRNA expression of genes related to glycolysis (GK and PK) and gluconeogenesis (G6Pase, FBPase, and PEPCK) in the liver of triploid rainbow trout fed the experimental diets for 80 days. Values are expressed as means ± S.E.M. ($n = 4$). Statistical significance was evaluated using one-way ANOVA, followed by Tukey's test. Values with different letters on columns statistically differ at $P < 0.05$.

regulation of hepatic lipogenic by dietary lipid is low controlled in rainbow trout same as described by Anne et al. ([32]) and Ducasse-Cabanot et al. ([41]). As the rate-limiting enzyme of TAG catabolism, HSL catalyzes the hydrolysis of TAG into DAG and DAG into MAG [42]. Then, the catabolism of fatty acids can occur by β-oxidation in both mitochondria and peroxisomes [43]. The present results suggested that the HL group could enhance the catabolism of TAG through elevating gene expression of HSL and the β-oxidation in peroxisomes (ACO). The ML group increased lipid β-oxidation in mitochondria (CPT1) compared with the LL group. A similar result of diploid rainbow trout found that the gene expression of ACO and CPT1 was upregulated in the liver of rainbow trout fed with the diet supplement lipid with 230 g kg^{-1} compared with that with 100 g kg^{-1} [44]. Contrary to lipolysis, glycolysis (GK and PK) is stimulated when fish are fed with limited lipids. Thus, it is reasonable to suppose that triploid rainbow trout could switch the energy source under different nutritional conditions. The results are conflict with that in diploid rainbow trout, in which no effects on the molecular levels for glycolysis enzymes (GK, 6PFK1, and PK) and the expression of genes coding for key enzymes of the gluconeogenic pathway (G6Pase and PEPCK) were significantly enhanced in the liver of fish fed with high diet lipid [41]. At present, we have no clear explanation for the discrepancy of the key genes involved in carbohydrate metabolism except for the difference in ploidy of fish.

PPARs are generally fatty acid sensors, responding to increased cellular fatty acid levels or their metabolites which arise as a result of changes in nutritional status and energy metabolism [45]. PPARα and PPARβ are responsible for regulating fatty acid β-oxidation [46]. PPARα activates lipid catabolism by regulating the expression of target gene encoding enzymes involved in the peroxisomal and mitochondrial β-oxidation of FAs, mainly in the liver [47]. The results of this study seem to confirm this conclusion; both PPARα and ACO gene expression reaches the highest value in fish fed with the highest dietary lipid level group. Elevated gene expression of PPARα was also found in fish fed HFD [48, 49] but not always [44]. Activation of PPARγ leads to increased TAG accumulation in muscle and liver by affecting transcription rates of a variety of lipogenic target genes such as FABP, CD36, LPL, ACC, and FAS [50]. The present results found that only higher gene expression levels of PPARγ, LPL, and FABPs were found in fish fed with relatively high lipid groups, suggesting that lipid uptake but not lipogenesis (ACC, FAS, G6PDH, and DGAT2) was stimulated. The relationship between PPAR expression, its functionality, and thus its role in controlling the expression of target genes is complicated and unclear at present [43]. Further research is still needed.

In conclusion, both viscera and muscle are the main lipid deposition sites for subadult triploid rainbow trout, which can maintain lipid homeostasis under various dietary lipid levels by regulating lipid uptake and switching energy supply between glycolysis and β-oxidation.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

All coauthors have seen and agree with the contents of the manuscript.

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