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Growth Performance, Lipid Deposition and Hepatic Lipid Metabolism Related Gene Expression in *Schizothorax prenanti* fed with Dietary Acidolysis-Oxidized Konjac Glucomannan Supplementation

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Abstract

The present study was conducted to investigate the effects of dietary acidolysis-oxidized konjac glucomannan (A-OKGM) (0, 0.4, 0.8 and 1.6%) supplementation on the growth performance, lipid metabolism of *Schizothorax prenanti*, a commonly cultured freshwater fish in inland China. The 1.6% A-OKGM diet significantly improved weight gain (WG) ($P<0.05$). Triglycerides (TG) content in both serum and liver was significantly increased when diets supplemented with A-OKGM, regardless of inclusion level ($P<0.05$). The total cholesterol (TC) and LDL-cholesterol (LDL-C) levels in serum were significantly increased when fish fed with 0.4%, 1.6% A-OKGM diets ($P<0.05$). However, only 1.6% A-OKGM diet significantly increased HDL-cholesterol (HDL-C) level ($P<0.05$). Dietary 1.6% A-OKGM supplementation had a significant effect on glucose level in the serum ($P<0.05$). However, the activities of phosphoenolpyruvate carboxykinase (PEPCK) and hexokinase (HK) were significantly decreased by both 0.8% and 1.6% A-OKGM diets ($P<0.05$). A-OKGM diets significantly affected glucose-6-phosphate dehydrogenase (G6PD) and total lipase activity of *Schizothorax prenanti*, regardless of inclusion level ($P<0.05$). The lipid content in the liver was significantly increased by A-OKGM diets, regardless of inclusion level ($P<0.05$). Moreover, A-OKGM diets significantly up-regulated the mRNA levels of lipogenic genes (PPARγ, FAS, ACCa, FABP, GPDH and HMGCR), regardless of inclusion level ($P<0.05$). The expression of CPT1 in fish fed with 1.6% A-OKGM diet was significantly lower than that of control group ($P<0.05$). These results encourage conducting further research on the administration of A-OKGM and other prebiotics in *Schizothorax prenanti*.

Keywords: *Schizothorax prenanti*; Acidolysis-oxidized konjac glucomannan; Growth performance; Lipid biosynthesis; Lipid metabolism

Abbreviations: HK: Hexokinase; PEPCK: Phosphoenolpyruvate Carboxykinase; SREBP-1: Sterol Regulatory Element-Binding Protein-1; PPARα: Peroxisome Proliferator Activated Receptor α; PPARγ: Peroxisome Proliferator Activated Receptor γ; FAS: Fatty Acid Synthase; ACCa: Acetyl-Coa Carboxylase a; FABP: Fatty Acid Binding Protein; HL: Hepatic Lipase; LPL: Lipoprotein Lipase; CPT1: Carnitine Palmitoyl Transferase 1; G6PD: Glucose-6-Phosphate Dehydrogenase; GPDH: Glycerol-3-phosphate Dehydrogenase; HMGCR: 3-Hydroxy-3-Methylglutaryl-CoA Reductase

Introduction

Prebiotics are non-digestible feed ingredients that beneficially affect the host by stimulating the growth of the host, and it also promoted activity of specific microorganism existed in the gastrointestinal tract, which have beneficial effects on host [1,2]. Prebiotics are also bioactive component [3] that provides not only nutrients but also oligosaccharides and polysaccharides, which improve growth performance and increase fish production [4,5]. Konjac glucomannan (KGM) is a potential prebiotic which is available in the tuber of *Amorphophallus konjac*. Because of its physical and chemical properties, the KGM have been widely used in food, pharmaceuticals, and chemical engineering industries [6]. Recently, the application of KGM is mainly focused on film preparation [7,8]. However, KGM is difficult to be used as a feed additive in aquafeeds industry due to its high molecular weight (500-2000 kDa) and viscosity. So, we prepared oxidized konjac glucomannan (OKGM) which is oxidatively derived from KGM. OKGM possess higher purity and lower molecular weight than that of KGM. Nonetheless, the molecular weight of OKGM is still high. Therefore, it is necessary to prepare low-molecular-weight OKGM to reduce its molecular weight and improve its bioactivity. In our study, hydrochloric acid (HCl) and hydroperoxide (H_2O_2) were used as degradation reagents to obtain acidolysis-oxidized konjac glucomannan (A-OKGM). The molecular weight of A-OKGM (9.8 kDa) was determined in our previous study [9]. Up to date many researchers have studied the effects of prebiotics on aquatic animals [10-13], and no information is available concerning the effects of A-OKGM on fish. *Schizothorax prenanti*, also known as 'ya fish' (Cypriniformes, Cyprinidae, Schizothoracinae), is an endemic cold fish in southwest part of China. It mainly reproduces in Yangtze River. Recently, *S. Prenanti* breeding industry is developing rapidly due to its delicious meat and high market value. However, poor growth performance of *S. Prenanti* has caused severe economic loss in some fish farms. Zhang et al. [14] reported that the growth performance of *S. Prenanti* was improved by the diets supplemented with 8000 mg/kg OKGM. In this regard, we hypothesized that A-OKGM may possess more positive effect on growth performance of *S. Prenanti* than OKGM did. Moreover, fish use lipid as energy for growth preferentially. Therefore, the aim of present study was to explore the effects of dietary A-OKGM on the growth performance and lipid metabolism of *S. Prenanti*. We investigated the expression of twelve important genes involved in lipid metabolism in liver, including *CPT1, LPL, ACCa, FAS, G6PD, FABP, GPDH, HL, PPARα, PPARγ, HMGRC* and *SREBP-1*, and activity of four

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enzymes involved in glucose and lipid metabolism, including PEPCK, G6PD, TLP, HK, and the content of LDL-C, HDL-C, TC, TG, Glu. To our best knowledge, our research is the first to explore the effect of A-OKGM on the lipid deposition and metabolism of *S. Prenanti* at both enzymatic and molecular level. These findings have profound implication in the application of A-OKGM in aquaculture field.

Materials and Methods

Preparation of A-OKGM

50 g KGM (purchased from PaiTe konjac biological technology co. Ltd, the purity is 95%) was dissolved in 250 ml 40% (v/v) ethanol solution, with stirring for 5 min at 180 r/min at 40°C. The pH value of solution was set to 4.3 using 10% (v/v) HCl. To initiate the reaction, 8.75 ml H_2O_2 (30% v/v) was added into the solution in 30 min at 10 min intervals. The reaction last for 4 h, and was terminated by adding Na_2SO_3 (1 mol/L). After the reaction, the pH value of the solution was set to 7 using 10% (v/v) NaOH, then filtrated with vacuum filter. The moisture matter was washed three times with 90% (v/v) ethanol, and filtrated 3 times individually. The moisture matter was subsequently dried at 90°C in the drum wind drying oven. Then, sample was grinded until it can be through 120 mesh sieve, and we got the OKGM. 50 g OKGM (obtained from the above step) was suspended in the solution containing 350 ml anhydrous ethanol and 100 ml concentrated HCl (36% v/v). The suspension was magnetically stirred for 2 h at room temperature, then filtrated with vacuum filter. The moisture matter was washed with 70% (v/v) ethanol until they became neutral. The samples were then left in a fume hood before being subjected to vacuum-drying at 30°C for 16 h, then we got the A-OKGM.

Diets

The experimental diets were supplemented with different levels (0.4%, 0.8% and 1.6%) of A-OKGM according to our previous study [9]. The diet without A-OKGM was used as control, Diets meet all nutritional requirements for *S. prenanti* and were manufactured using the formulation (Table 1).

Diets	Control	A-OKGM		
Formulation (g kg^{-1})	1	2	3	4
A-OKGM	0	4	8	16
Fish meal	420	420	420	420
Rapeseed oil	30	30	30	30
Soybean meal	210	210	210	210
Flour	200	200	200	200
Starch	100	96	92	84
Bran	10	10	10	10
Vitamin premix + choline*	5	5	5	5
Mineral premix†	10	10	10	10
$Ca(H_2PO_4)_2$	15	15	15	15
Total	1000	1000	1000	1000
Nutrition level (%)				
Crude protein	35.2	35.2	35.2	35.2
Crude lipid	8.19	8.19	8.19	8.19
TE (MJ/kg) ‡	16.53	16.46	16.39	16.31
Ca	2.02	2.02	2.02	2.02
P	1.52	1.52	1.52	1.52
Lys	2.87	2.87	2.87	2.87
Met+Cys	1.32	1.32	1.32	1.32

*Vitamin provides for per kg diet: VA 5000 IU; VD 1000 IU; VE 30 IU; VK 2.5 mg; VB_1 5 mg; VB_2 8 mg; VB_6 7 mg; VB_{12} 0.01 mg; Niacin 30 mg; pantothenic acid 25 mg; folic acid 0.5 mg; biotin 0.2 mg; VC 35 mg; Inositol 50 mg; choline chloride 700 mg.
†Mineral provides for per kg diet: Mn 10 mg; Zn 30 mg; Fe 60 mg; Cu 3 mg; I 1 mg; Se 0.2 mg.
‡Total energy (TE) is calculated value. Other nutrient levels are measured values.

Table 1: Formulation of experimental diets.

Feeding experiments

Three hundred fish were transferred from a local farm (Yuquantown, Tianquan, Yaan, Sichuan, China) to the laboratory of Functional Food of Sichuan Agriculture University where they were allowed to adapt to the environment for three weeks in 15 fiberglass tanks (50 cm × 70 cm × 40 cm), and thereafter 240 healthy and robust fish (initial weight 70.12 ± 1.56 g) were selected for experimental use and they were reared in 12 tanks (20 fish per tank) at the temperature of 15-25°C, under natural photoperiod (12L,12D). Each diet was assigned to three tanks in a completely randomized Manner. The diets were fed to the fish at fix ration (2% of the total fish weight/per tank) three times daily. The tanks were aerated to maintain the dissolved oxygen levels at saturation. Fecal matter was quickly removed during the experiment, and water in each tank was replenished 100% daily. The experiment was carried out for 8 weeks. All procedures above were approved by Laboratory Animal Management Committee of Sichuan Province (permit number SYXK2015-196, 22 July 2015).

Sampling

At the end of the experiment, 24 h after the last feeding, fish were killed by a blow to the head. All fish were counted and weighed to determine the survival rate and weight gain. A total of ten fish per tank were randomly collected and stored frozen (-80°C) for proximate composition analysis. Blood samples (2 fish/per tank) were collected from the caudal vein before the dissection of fish. After the blood samples were clotted, they were centrifuged at 3000 g for 10 mins at 4°C. The supernatants were collected as serum which were used to analyze serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Then, fish (2 fish/per tank) were dissected on ice to obtain livers for determination of lipid content and enzymes activity. For RNA extraction, the livers of two fish from each tank were dissected and pulverized with mortars under liquid nitrogen.

Sample analysis

Crude lipid, crude protein, ash and moisture contents of fish body samples were analyzed using the established standard methods. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were analyzed with commercial kits (Nanjing Jiancheng bioengineering Institute, China) according to manufacturer's instruction. The TC and TG content in the liver were determined using the same kits used for serum. The total lipid content of liver was determined according to the method of Folch et al. [15].

Enzyme activity analysis

Liver samples were homogenized in 10 vol (w/v) of ice-cold physiological saline and centrifuged at 6000 g at 4°C for 20 min and the supernatant was collected for enzymes activity analysis. The activities of G6PD, Total lipase, HK and PEPCK were determined by four commercial fish-specific kits (Shanghai Yaji biotechnology Institute, China) according to manufacturer's instruction.

Total RNA extraction and cDNA synthesis

The total RNA was extracted from liver samples using RNA isolater

reagent (VazymE, Nanjing, China) according to the manufacturer's instruction. The RNA was treated with RNase-free H_2O to the final volume of 20 μL and the integrity of RNA was tested by electrophoresis in 1.0% agarose gel. Moreover, the quantity of isolated RNA was later determined by measuring the absorbance at 260 and 280 nm. After that, 6 μl of the total RNA was used to synthesize the first-strand cDNA using HiScript®1st Strand cDNA Synthesis Kit (VazymE, Nanjing, China) according to the manufacturer's instruction, and the cDNA was stored at -80°C for later use.

RT-qPCR

The CFX96 real-time PCR (Bio-Rad) and SYBR Green (VazymE, Nanjing, China) were performed to analyze the expression of genes involved in lipid metabolism, and all the primers were designed from the existed sequences of *Carassius auratus* (KF767100.1), *Cyprinus carpio* (KF844251.1), *Danio rerio* (AB183467.1). Table 2 listed all the primers used in our study. The primer of β-actin was designed as housekeeping gene from *S. prenanti* β-actin sequence (JQ013000). The RT-PCR was performed with final volume of 10 μl, each reaction contained 5 μl of AceQ qPCR SYBR Green Master Mix (VazymE, Nanjing, China), 0.25 μl of each primer (10 μM), 1 μl of cDNA and 3.5 μl RNase-free H_2O. The amplification of each gene consisted of 95°C for 3 min, followed by 39 cycles of 95°C for 10 s and Annealing temperature for 30 s. After each run, we generated the melting curves which were used to ensure the specificity of primers by increasing the temperature from 65°C for 5 s to 95°C, at 0.5°C/s. Gene expression results were analyzed using the $2^{-\Delta\Delta Ct}$ method. Moreover, the products of RT-PCR were tested by electrophoresis in 2.0% agarose gel (supplement materials).

Calculations and statistical analysis

Survival rate (SR,%)=$100 \times F_N/I_N$

Weight gain (WG,%)=$100 \times (W_t - W_0)/W_0$

Specific growth rate (SGR,%/day)=$(\ln W_t - \ln W_0)/T \times 100$

Where F_N is the final fish number, I_N is the initial fish number, W_t is the wet final weight, W_0 is the wet initial weight, and T is the experimental period. All data is presented as the mean ± S.E. Data was subjected to one-way ANOVA and Duncan's multiple range tests using the software SPSS 16.0 statistical software package (SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P<0.05$.

Results

Growth performance

Survival rate of fish ranged from 95 to 100% and was non-significantly different among the dietary treatments (Table 3). Fish fed with 1.6% A-OKGM diet exhibited significantly higher weight gain and specific growth rate compared with control group ($P<0.05$).

Proximate composition of fish body and lipid content in tissue,

The final body compositions of fish were significantly affected by dietary A-OKGM (Table 4). The crude protein content was significantly increased in fish fed with 1.6% A-OKGM diet when compared with control group ($P<0.05$). The crude lipid content in fish fed with both 0.4% and 1.6% A-OKGM diets was significantly higher than that in control group ($P<0.05$). However, there were no significant differences

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (bp)	Annealing temperature
SREBP-1	GTGGTTGTGTCACCCTCTG	GACTGTGTTGTGGCTCCTG	285	58.2°C
PPARα	CTCCGCCTTTCGTCATCC	CAGTAGAGTCACCTGGCGTTG	242	58.8°C
PPARγ	GCCTCGGGCTTCCATTAC	CTTGTTGCGACTCTTCTTGT	132	58.2°C
FAS	CAGTTGTGAAGACTCGGC	CCTCTGTTCCAGTAGTGTATG	159	56°C
LPL	GCAACAACACTACCCTACATC	GGTGAGAAGACCAGCAAT	166	56°C
ACCa	GCAACCACATCTTCCTCAAC	CGGAGTTTCCACAGACGA	112	58°C
CPT1	CCTCTCCCCACCAAACTACA	GCCTTTCCCAAACTCATCAA	135	52.4°C
G6PD	TCGTGGAGAAGCCTTTTGG	AACCACACACGCCACACTG	200	59.4°C
FABP	CACCCGTCAGGTTGGCA	TGTCAGAGTTAGGCTGTTGTCA	265	58.8°C
HL	CACACGAATAGTGGGACAGG	AGGGTAAATGTATGGATGGC	268	56.2°C
GDPH	CTCATTTTCGTTATCCCTCAC	ACATTCCCACAGCATCCGTTT	85	58.8°C
HMGCR	GAACCGAGACCTGTGGAAGA	TGTAAGCTGGGATGTGCTTG	118	56.8°C
β-actin	GATTCGCTGGAGATGATGCT	CGTTGTAGAAGGTGTGATGCC	218	55.8°C

Table 2: Primers used in real-time PCR analysis.

Diets	control	A-OKGM 0.4%	A-OKGM 0.8%	A-OKGM 1.6%
IBW (g/fish)	70.29 ± 0.32	70.10 ± 0.40	70.23 ± 0.63	69.86 ± 0.53
WG (%)	3.01 ± 0.61^a	4.07 ± 0.86^a	4.50 ± 0.94^a	11.17 ± 0.19^b
SGR (%/d)	0.05 ± 0.01^a	0.07 ±0.01^a	0.07 ± 0.02^a	0.18 ± 0.00^b
SR (%)	98.33 ± 1.67	98.33 ±1.67	95.00 ± 2.87	100
IBW, Initial mean body weight; WG, Weight gain; SGR, Specific growth rate; SR, Survival rate. The observed values were expressed as mean ± S.E, n=3. Mean values with different superscripts are significantly ($P<0.05$) different from each other.				

Table 3: Growth performance of *S. prenanti* during the feeding period.

Diets	Control	A-OKGM 0.4%	A-OKGM 0.8%	A-OKGM 1.6%
Moisture	78.36 ± 0.18	78.35 ± 0.14	78.55 ± 0.32	78.34 ± 0.13
Crude protein	15.20 ± 1.25^a	16.34 ± 1.35^{ab}	16.74 ± 0.21^{ab}	18.59 ± 0.15^b
Crude lipid	1.72 ± 0.02^a	2.12 ± 0.13^b	1.91 ± 0.09^{ab}	2.89 ± 0.09^c
Ash	1.24 ± 0.02	1.26 ± 0.03	1.32 ± 0.03	1.28 ± 0.04
The observed values were expressed as mean ± S.E, n=3. Mean values with different superscripts are significantly ($P<0.05$) different from each other.				

Table 4: Proximate composition (% wet weight) of the whole body of *S. prenanti* feed with diets containing different A-OKGM levels.

in the moisture and ash contents between the four diet groups (P>0.05). Dietary A-OKGM significantly affected serum lipid levels (Table 5). The serum TG level was significantly increased by dietary A-OKGM supplementation, regardless of inclusion level (P<0.05). Fish fed with 0.4% and 1.6% A-OKGM diets showed significantly higher serum TC level compared with control group (P<0.05). The serum LDL-C level was highest in fish fed with 0.4% A-OKGM diet, but the highest serum HDL-C level was appeared in 1.6% A-OKGM diet. The serum LDL-C, HDL-C ratio in fish fed with 0.4% and 1.6% A-OKGM diets was significantly different from control group (P<0.05). The serum GLU level in fish fed with 1.6% A-OKGM diet was significantly higher compared with control group (P<0.05). Dietary A-OKGM supplementation significantly increased hepatic total lipid, TG and TC contents, regardless of inclusion level (P<0.05) (Table 5), and the highest total lipid and TG value was observed in fish fed with 1.6% A-OKGM diet. However, the highest TC value appeared in 0.8% A-OKGM diet group.

Diets	Control	A-OKGM 0.4%	A-OKGM 0.8%	A-OKGM 1.6%
Serum				
TG (mmol/l)	1.58 ± 0.06^{a}	2.9 8 ± 0.21^{b}	2.89 ± 0.17^{b}	3.48 ± 0.18^{c}
TC (mmol/l)	6.83 ± 0.22^{a}	9.14 ± 0.14^{b}	7.46 ± 0.29^{a}	9.02 ± 0.22^{b}
LDL-C (mmol/l)	1.03 ± 0.07^{a}	2.65 ± 0.10^{c}	1.26 ± 0.11^{a}	1.64 ± 0.02^{b}
HDL-C (mmol/l)	3.04 ± 0.11^{a}	3.30 ± 0.07^{ab}	3.24 ± 0.24^{ab}	3.62 ± 0.04^{c}
LDL-C: HDL-C	0.35 ± 0.03^{a}	0.81 ± 0.05^{c}	0.39 ± 0.02^{ab}	0.45 ± 0.01^{b}
GLU (umol/l)	33.54 ± 5.48^{a}	36.62 ± 3.05^{a}	39.69 ± 3.04^{a}	55.42 ± 3.23^{b}
Liver				
Total lipid (%)	8.70 ± 0.26^{a}	11.64 ± 0.60^{b}	10.61 ± 0.36^{b}	13.93 ± 0.26^{c}
TG (mmol/gprot)	0.56 ± 0.04^{a}	1.28 ± 0.08^{b}	1.18 ± 0.05^{b}	1.90 ± 0.04^{c}
TC (mmol/gprot)	0.23 ± 0.02^{a}	0.47 ± 0.00^{b}	0.54 ± 0.04^{b}	0.47 ± 0.05^{b}

TG, triglycerides; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; GLU, glucose.
The observed values were expressed as mean ± S.E, n=6. Mean values with different superscripts are significantly (P<0.05) different from each other.

Table 5: Lipid profiles in serum, liver of *S. prenanti* fed diets with different A-OKGM levels.

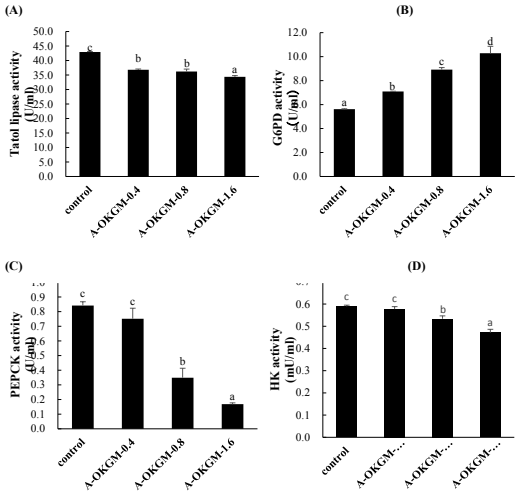

Figure 1: Effects of dietary A-OKGM concentrations on the activities of enzymes ((A) total lipase (TLP), (B) glucose-6-phosphate dehydrogenase (G6PD), (C) phosphoenolpyruvate carboxykinase (PEPCK), (D) hexokinase (HK)) involved in lipid and glucose metabolism in the liver of *S. prenanti* after dietary A-OKGM supplementation for 8 weeks. Values are means (n=6), with their standard errors represented by vertical bars. ^{a,b,c,d} Mean values within a tissue with unlike letters were significantly different (P<0.05).

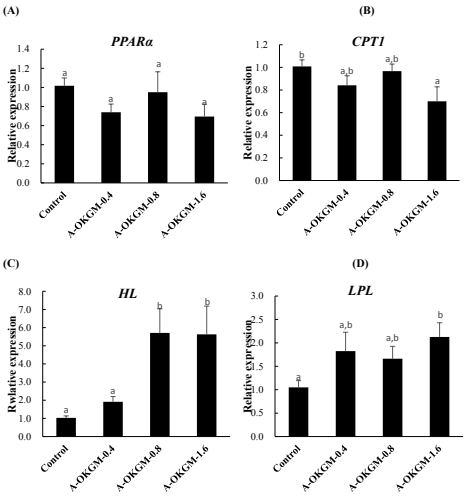

Figure 2: Results of quantitative real-time PCR analysis carried out for genes involved in lipolysis ((A) *PPARα*, (B) carnitine palmitoyl transferase 1 (*CPT1*), (C) hepatic lipase (*HL*) and (D) lipoprotein lipase (*LPL*)) in the liver of *S. prenanti* after dietary A-OKGM supplementation for 8 weeks. Values are means (n=6), with their standard errors represented by vertical bars. ^{a,b} Mean values within a tissue with unlike letters were significantly different (P<0.05).

Enzyme activity

The effects of dietary A-OKGM supplementation on the activities of Total lipase, G6PD, PEPDK and HK in the liver of *S. prenanti* are shown in Figure 1. The activity of Total lipase was significantly decreased by the A-OKGM diets, regardless of inclusion level (P<0.05). The activities of PEPCK and HK in fish fed with 0.8% and 1.6% A-OKGM diets were significantly lower than that in control group (P<0.05). However, dietary A-OKGM supplementation significantly enhanced the activity of G6PD, regardless of inclusion level (P<0.05).

Gene expression involved in lipolysis

The effects of dietary A-OKGM supplementation on the expression of lipolytic genes in the liver of *S. Prenanti* are shown in Figure 2. In the liver, the expression of *PPARα* didn't reach significant level (P>0.05). The mRNA level of *CPT1* in fish fed with 1.6% A-OKGM diet was significantly down-regulated compared with control group (P<0.05). However, both 0.8% and 1.6% A-OKGM diets significantly up-regulated the expression of *HL* (P<0.05). Fish fed with 1.6% A-OKGM diet showed significantly higher *LPL* expression than that in fish fed with control diet (P<0.05).

Gene expression involved in lipogenesis

The effects of dietary A-OKGM supplementation on the mRNA levels of lipogenic genes in the liver of *S. Prenanti* (Figure 3). In the liver, the expressions of *SREBP-1*, *PPARγ*, *ACCa*, *FAS*, *FABP*, *GPDH*, *G6PD* and *HMGCR* were up-regulated by A-OKGM diets. Moreover, the mRNA levels of *PPARγ*, *ACCa*, *FAS*, *GPDH*, *FABP* and *HMGCR* were significantly enhanced by the A-OKGM supplementation, regardless of inclusion level (P<0.05). However, only 0.4% A-OKGM diet significantly up-regulated the expression of *SREBP-1*, and the 1.6% A-OKGM diet significantly up-regulated the expression of *G6PD* (P<0.05).

Discussion

It is clear from the present study that dietary supplemented with A-OKGM possessed a positive effect on growth performance of *S.*

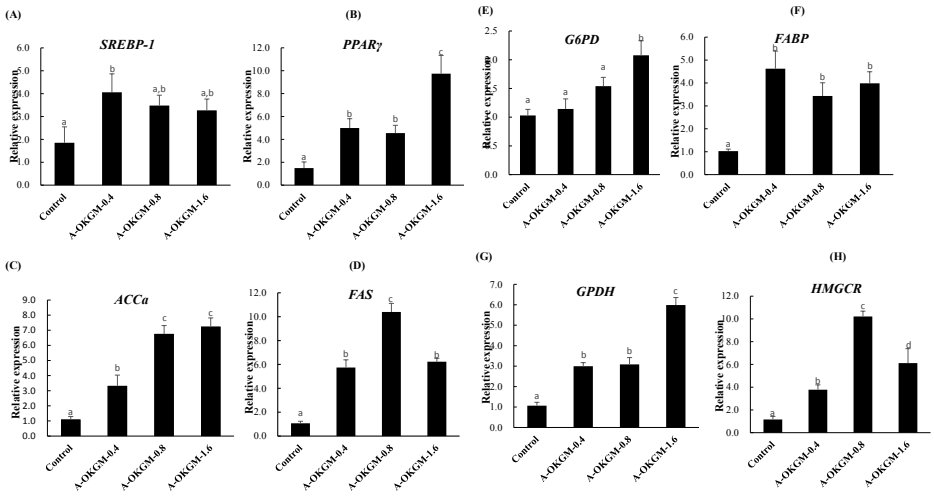

Figure 3: Results of quantitative real-time PCR analysis carried out for genes involved in lipolysis ((A) *SREBP-1*, (B) *PPARγ*, (C) acetyl-CoA carboxylase a (*ACCa*), (D) fatty acid synthase (*FAS*), (E) glucose-6-phosphate dehydrogenase (*G6PD*), (F) fatty acid binding protein (*FABP*), (G) glycerol-3-phosphate dehydrogenase (*GPDH*), (H) 3-hydroxy-3-methylglutaryl-CoA reductase (*HMGCR*)) in the liver of *S. prenanti* after dietary A-OKGM supplementation for 8 weeks. Values are means (n=6), with their standard errors represented by vertical bars. ^{a,b,c,d} Mean values within a tissue with unlike letters were significantly different (P<0.05).

prenanti, with fish fed with the diet supplemented with 1.6% A-OKGM exhibiting the greatest weight gain. The improved growth observed in the present study was similar to that reported in rainbow trout [16], common carp [17], sea bream [18]. However, the growth-promoting effect of dietary prebiotics supplementation was not observed in Atlantic salmon [19] and red drum [20]. The body composition of *S. Prenanti* was affected by the A-OKGM diets, the crude lipid content in fish fed with both 0.4% and 1.6% A-OKGM diets was significantly higher than that in control group. Similar results were also found in giant sturgeon juvenile [21] and common carp fry [22]. Moreover, the 1.6% A-OKGM diet significantly enhanced the crude protein content. Fish growth is mainly achieved through protein accumulation [23]. Such result is consistent with the previous studies where *Lates calcarifer* fed with 20 g kg^{-1} inulin showed the higher crude protein when compared with control group [24]. It has been well established that prebiotics can change gut morphology or gut microbial community of fish. Hence, the improved growth performance in our present study can be caused by the following reasons. First, some beneficial commensal bacteria can ferment prebiotics to produce short chain fatty acids which play an important role in improving host growth performance [25]. Second, growth is closely related with nutrient uptake which positively correlates with microvillus height [26]. The improvement of microvillus morphology was found in rainbow trout [27], cobia [28] after fish were fed with prebiotics. Future studies will be guaranteed to elucidate the underlying mechanism of the improved growth performance of *S. Prenanti* after the administration of A-OKGM.

Compared with mammals, fish, the largest vertebrate group, have lower capabilities to use carbohydrates as energy, thus, main source of energy for growth and movement is lipid [29]. It's well known that the liver and muscle of fish have the ability to store the lipid [30,31]. Lipid metabolism represents a complex process, which include oxidation, accumulation and transport [32]. In our present study, the increased lipid content was observed in the liver after administrated with A-OKGM diets for 8 weeks, particularly, the 1.6% A-OKGM diet. Fish lipid synthesis and oxidation determine the lipid accumulation level [33]. Moreover, lipid synthesis is regulated by different kinds of lipogenic enzymes, which is controlled primarily by SREBP-1 [34] and secondarily by PPARγ [35] in the liver. The mRNA levels of *SREBP-1* and *PPARγ* in present study were positively related with the lipid content, which were significantly up-regulated by the A-OKGM diets. However, the expression of *PPARα* was down-regulated, but didn't reach significant level. PPARα plays an important role in stimulating lipid oxidation due to the fact that it can induce β-oxidation by up-regulating key enzymes such as CPT1 [36,37]. The expression of *CPT1* in this study was also down-regulated in liver. Similar results were found in the mammal [38]. Taken together, these findings indicated that the increased hepatic lipid deposition caused by A-OKGM diets was attributed to promotion of lipid synthetic ability and to depression of lipid oxidation ability.

As triglycerides is a major component of lipid, its content in both serum and liver was significantly increased by A-OKGM diets. The observation is in accordance with that by Zhao et al. [39], who reported that TG content in *Litopenaeus vannamei* fed with 250 mg kg^{-1} β-1,3-glucan diet was significantly higher than that observed in other diets. In our view, the augment of TG content in the liver is mainly achieved through *de novo* lipogenesis (DNL) pathway. DNL is a highly regulated, complex process through which nutrition consumed in excess of oxidative needs could be stored as lipid in the liver of vertebrates [40]. On the one hand, the activation of the pentose phosphate pathway, at both transcriptional and enzymatic level, was observed when *S. Prenanti* fed with A-OKGM diets. This was revealed by strong up-regulation of *G6PD* expression and enhanced G6PD activity, the main enzyme of this pathway. The NADPH (reduced form of nicotinamide adenine dinucleotide phosphate) which is needed for a variety of biosynthetic processes involved in lipid metabolism [41,42] or carbon backbones (as one of the main precursors of acetyl-CoA) were provided through this pathway for DNL. However, the activity of HK which is the rate limiting enzyme of glycolysis was significantly decreased in fish fed with 0.8% and 1.6% A-OKGM diets after the feeding trial. This indicated that the glycolysis pathway was not activated by the A-OKGM diets, at least at enzymatic level. On the other hand, the elevated expressions of *ACCa*, *FAS* and *GPDH* were also observed in this study. The ACCa can catalyze more acetyl-CoA produced through pentose phosphate pathway to malonyl-CoA, which will be used to synthesize FA (fatty acid) [43]. At the same time, fatty acid synthesis was promoted by the largely up-regulated expression of *FAS* involved

in fatty acid synthesis [44]. Subsequently, *de novo* synthesis of FA is up-regulated and contribute to increasing TG synthesis mediated by GPDH [45]. Besides, the A-OKGM diets also significantly promoted the expression of *FABP* in liver of *S. Prenanti*. FABP is a member of large multigene family of intracellular lipid-binding proteins (iLBPs), and plays crucial roles in fatty acid transportation, sequestering and metabolism [46]. Although some researches have studied the function of FABP in fish [47,48], compared with the mammals, the function of fish FABP in lipid metabolism is not clearly understood. All in all, accelerated pentose phosphate pathway provides more substrate (acetyl-CoA and NADPH) for FA, and the increased *SREBP1*-mediated expression of lipogenic genes (*ACCa* and *FAS*), intracellular FA transport gene (*FABP*) and *GPDH* promised the FA would be efficiently synthesized and esterified to form TG in hepatocytes. However, TG synthesis is a complex process that still need further investigation in *S. Prenanti* in the future. In addition, the activity of the Phosphoenolpyruvate carboxykinase (PEPCK) in *S. Prenanti* fed with A-OKGM diets, especially, 1.6% A-OKGM diet, was lower than that in the control group, and the PEPCK is the rate-limiting enzyme involved in the gluconeogenesis. We hold the point that this phenomenon may be cause by the increased level of glucose in the serum. *S. Prenanti* fed with 1.6% A-OKGM diet show the highest glucose level, and the relatively high glucose level may inhibit the activity of PEPCK. Hepatic lipase (HL) is a lipolytic enzyme that plays a critical role in clearing TG from the plasma [49]. Similar to the HL, Lipoprotein lipase (LPL) plays a key role in lipid metabolism by hydrolyzing triglycerides transported in the bloodstream to very low-density lipoproteins [50]. HL Together with LPL, is responsible for the clearance of TG from the circulation. In our present study, the activity of Total lipase which is sum of the HL and LPL was significantly lower than that in control group. The decreased Total lipase activity is another reason for the elevated TG content in both serum and liver. However, it is worth noting that both *HL* and *LPL* expressions were up-regulated after the administration of A-OKGM. We hold the point that the up-regulated *LPL* and *HL* gene expressions maybe is too weak to influence the activity of Total lipase or the effects of dietary A-OKGM on *LPL* and *HL* gene expressions could decrease at a post-transcriptional level.

In human and some aquatic animals, excess lipids deposition is harmful for health [51,52]. However, relatively high blood lipids level can also be caused by the high requirement of energy for growth. This is revealed by the fact that the promoted growth in A-OKGM groups positively correlated with high blood lipids content. Therefore, present finding suggested that A-OKGM can increase lipid utilization to release energy which was used for growth.

As cholesterol is a major component of cell membranes, it play an important role in regulating membrane fluidity and permeability [53]. Besides, it's also the precursor molecule of other steroids, such as bile salts, steroid hormones, and vitamin D [54]. In this study, the serum TC level was significantly increased by the 0.4% and 1.6% A-OKGM diets. Besides, the TC content in the liver was also significantly higher than that in control group, regardless of the A-OKGM level. Similar results were found in studies on *Oreochromis niloticus* [55] and *huso huso* [56] after the administration of inulin. Cholesterol biosynthesis is controlled by a feedback mechanism with HMGCR as the key enzyme. HMGCR is highly expressed in the liver [57], and can converts HMG-CoA to mevalonate which is the key precursor for the cholesterol synthesis [58]. Thus, the expression of *HMGCR* gene contributes to the overall cholesterol status. The present study showed that the expression of *HMGCR* was significantly up-regulated by the A-OKGM diets, regardless of the inclusion level. The elevated *HMGCR* expression is responsible for the increased TC content. Similar to mammals, fish lipoproteins can be classified as VLDL, intermediate-density lipoprotein, LDL and HDL [59]. HDL carries cholesterol from the peripheral tissues to the liver, whereas LDL carries cholesterol from the liver to the peripheral tissues. Thus, the LDL-C, HDL-C ratio can be used as a sign of cholesterol transport [60]. In our study, the dietary A-OKGM supplementation significantly enhanced the serum LDL-C, HDL-C ratio in fish fed with 0.4% and 1.6% A-OKGM diets. These indicated that A-OKGM diets may increase the ability of carrying the cholesterol from peripheral tissue to liver of *S. Prenanti*.

While prebiotics can be used to control hyperlipidaemia caused by diabetes and other conditions, it cannot decrease lipid level of healthy subjects [61]. Moreover, 0.5% scFOS diet increased plasmatic total lipid content of gilthead sea bream [62]. The similar changes in the lipid content were observed in present study, and the alteration of lipogenic genes expression and enzymes activities is responsible for the increased lipid content in serum and liver. It has been reported that short chain fatty acids (SCFAs), such as propionate and acetate, may be involved in the process of lipid metabolism by inhibiting fatty acids synthesis or providing substrates for lipogenesis [63,64]. SCFAs is produced by intestinal microbial flora when they fermented the prebiotics. Thus, the changes of lipogenic genes expression and enzymes activities reported in the present study might be related to SCFAs producing bacteria existed in fish intestine tract and that deserves to be further investigated.

Conclusion

In summary, the growth performance of *S. Prenanti* was improved by A-OKGM diets. Besides, dietary A-OKGM supplementation was found to increase lipid content in the present study. The increased lipid content in the liver could be attributed to the up-regulation of the mRNA levels of lipogenic genes (such as *SREBP-1*, *PPARγ*, *FAS*, *ACCa, FABP, GPDH, G6PD and HMGCR*) and to the increased activity of lipogenic enzyme (G6PD) and to the depressed activity of lipolytic enzyme (TLP) and to the down-regulation of the mRNA levels of the lipolytic genes (*PPARα* and *CPT1*). A-OKGM, as prebiotics, its effect on lipid metabolism in *S. Prenanti* is opposite to that in mammals [65,66]. Hence, the underlying mechanism needs to be determined in future studies.

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