Effects of Dietary Carbohydrate-to-Lipid Ratio on Growth Performance, Hepatopancreatic Metabolic Enzyme Activities, Fatty Acid Transport, and Toll-Like Receptor Pathway-Related Gene Expression in Macrobrachium nipponense (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary carbohydrate-to-lipid ratio on growth performance, hepatopancreatic metabolic enzyme activities, fatty acid transport, and Toll-like receptor pathway-related gene expression in Macrobrachium nipponense. Five isonitrogenous (crude protein content of 39%) experimental diets with carbohydrate-to-lipid ratios of 1.12 (CL1), 2.00 (CL2), 3.56 (CL3), 7.10 (CL4), and 24.12 (CL5) were formulated to conduct an 8-week feeding trial on M. nipponense [(0.104±0.003) g]. The experimental prawns were randomly divided into 5 groups, with 5 replicates per group and 50 individuals per replicate. The results showed: when the carbohydrate-to-lipid ratio increased from 1.12 to 7.10, the weight gain rate of M. nipponense showed no significant change ($P>0.05$); however, when the ratio further increased to 24.12, the weight gain rate was significantly reduced ($P<0.05$). There was no significant difference in survival rate among all groups ($P>0.05$). The hepatopancreatic glycogen content in the CL5 group was significantly higher than that in the CL4 group ($P<0.05$). The CL4 group exhibited the highest hepatopancreatic hexokinase (HK) and pyruvate kinase (PK) activities; its HK activity was significantly higher than that of the CL1 group ($P<0.05$), and PK activity was significantly higher than all other groups ($P<0.05$). Hepatopancreatic malondialdehyde (MDA) content, total antioxidant capacity (T-AOC), and catalase (CAT) activity showed no significant differences among groups ($P>0.05$); however, superoxide dismutase (SOD) activity in the hepatopancreas of CL1 and CL2 groups was significantly higher than that in CL4 and CL5 groups ($P<0.05$).

The expression levels of class B type I scavenger receptor (SR-BI) and fatty acid transport protein 4 (FATP4) genes in the hepatopancreas of the CL1 group were significantly higher than those in all other groups (P<0.05); however, the expression level of fatty acid binding protein 10 (FABP10) gene in the hepatopancreas of the higher carbohydrate-to-lipid ratio group (CL4 group) was significantly higher than that in the lower ratio groups (CL1, CL2, and CL3 groups) (P<0.05). The expression levels of Toll-like receptor 3 (TLR3), myeloid differentiation protein 88 (MyD88), interleukin-1 receptor-associated kinase 4 (IRAK4), tumor necrosis factor receptor-associated factor 6 (TRAF6), mitogen-activated protein kinase kinase kinase 7 (MAP3K7), and mitogen-activated protein kinase 14 (MAPK14) genes in the hepatopancreas of the CL3 group were significantly higher than those in all other groups (P<0.05). These results indicate that M. nipponense exhibits considerable adaptability to dietary carbohydrate-to-lipid ratios; however, an excessively high ratio (24.12) inhibits its growth and increases hepatic glycogen accumulation. Dietary carbohydrate-to-lipid ratio can regulate fatty acid transport and Toll-like receptor pathway-related expression in the hepatopancreas of M. nipponense.

Full Text

Effects of Dietary Carbohydrate to Lipid Ratio on Growth Performance, Hepatopancreas Metabolic Enzyme Activities and Expression of Fatty Acid Transport- and Toll-Like Receptor Pathway-Related Genes of Oriental River Prawn (Macrobrachium nipponense)

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Abstract

This study aimed to investigate the effects of dietary carbohydrate to lipid ratio on growth performance, hepatopancreas metabolic enzyme activities, and expression of fatty acid transport- and Toll-like receptor pathway-related genes in oriental river prawn (*Macrobrachium nipponense*). Five isonitrogenous (crude protein content: 39%) experimental diets with carbohydrate to lipid ratios of 1.12 (CL1), 2.00 (CL2), 3.56 (CL3), 7.10 (CL4), and 24.12 (CL5) were formulated and used in an 8-week feeding trial with oriental river prawns [initial body weight: (0.104±0.003) g]. The prawns were randomly divided into 5 groups with 5 replicates per group and 50 prawns per replicate. The results showed that no significant differences were observed in weight gain rate when the carbohydrate

to lipid ratio increased from 1.12 to 7.10 (P>0.05). However, when the carbohydrate to lipid ratio further increased to 24.12, the weight gain rate was significantly reduced (P<0.05). No significant differences were observed in survival rate among all groups (P>0.05). The hepatopancreas glycogen content in CL5 group was significantly higher than that in CL4 group (P<0.05). The highest activities of hexokinase (HK) and pyruvate kinase (PK) in hepatopancreas were observed in CL4 group; HK activity in CL4 group was significantly higher than that in CL1 group (P<0.05), while PK activity was significantly higher than all other groups (P<0.05). No significant differences were observed in hepatopancreas malondialdehyde (MDA) content, total antioxidant capacity (T-AOC), and catalase (CAT) activity among all groups (P>0.05), but hepatopancreas superoxide dismutase (SOD) activity in CL1 and CL2 groups was significantly higher than that in CL4 and CL5 groups (P<0.05). The expression levels of scavenger receptor class B type I (SR-BI) and fatty acid transport protein 4 (FATP4) genes in hepatopancreas were significantly higher in CL1 group than in all other groups (P<0.05). However, the expression level of fatty acid binding protein 10 (FABP10) gene in hepatopancreas was significantly higher in the higher carbohydrate to lipid ratio group (CL4) than in the lower carbohydrate to lipid ratio groups (CL1, CL2, and CL3) (P<0.05). The expression levels of hepatopancreas Toll-like receptor 3 (TLR3), myeloid differentiation primary response protein 88 (MyD88), interleukin 1 receptor associated kinase 4 (IRAK4), tumor necrosis factor receptor associated factor 6 (TRAF6), mitogen-activated protein kinase kinase kinase 7 (MAP3K7), and mitogen-activated protein kinase 14 (MAPK14) genes in CL3 group were significantly higher than those in all other groups (P<0.05). These results indicate that oriental river prawn has considerable adaptability to dietary carbohydrate to lipid ratio, but excessively high carbohydrate to lipid ratio (24.12) inhibits its growth and increases hepatic glycogen accumulation. Dietary carbohydrate to lipid ratio can regulate the expression of fatty acid transport- and Toll-like receptor pathway-related genes in the hepatopancreas of oriental river prawn.

Keywords: oriental river prawn (*Macrobrachium nipponense*); carbohydrate to lipid ratio; growth; antioxidant; lipid metabolism; immune

Introduction

Carbohydrates and lipids are important non-protein energy sources for aquatic animals. Compared with protein, they are not only inexpensive but also improve protein utilization efficiency. Consequently, research on the nutrition and physiology of carbohydrates and lipids has attracted increasing attention. Lipids serve as important sources of energy, essential fatty acids, phospholipids, and sterols, and maintain normal physiological activities, biological structure, and physiological functions of cell membranes. Appropriate levels of fat and essential fatty acids in animals can improve growth performance, antioxidant capacity, and immune response. However, excessively high lipid content in aquafeed may

cause problems such as inhibited growth, excessive fat deposition, and altered metabolic levels. Compared with lipids, carbohydrates represent a cheaper and more readily available energy source for aquatic animals, but insufficient or excessive carbohydrate levels may inhibit growth, affect metabolism, and weaken immune response. Therefore, an optimal dietary carbohydrate to lipid ratio is crucial for promoting growth, maintaining normal physiological functions, and ensuring health status in aquatic animals. Imbalanced dietary fat and carbohydrate content and ratios may affect metabolic pathways, prompting researchers to investigate optimal carbohydrate to lipid ratios that promote growth while maximizing economic benefits.

Previous studies have demonstrated that appropriate dietary carbohydrate to lipid ratios optimize growth performance in yellow catfish (*Pelteobagrus fulvidraco*) and improve growth performance and feed utilization in large yellow croaker (*Larmichthys crocea*), with similar results reported in tilapia (*Oreochromis niloticus*). However, when carbohydrate to lipid ratios continue to increase, tissue digestive enzyme activity is inhibited in common carp (*Cyprinus carpio*), antioxidant capacity decreases, and growth performance is affected. Conversely, when carbohydrate to lipid ratios decrease, whole body, carcass, and liver fat content as well as viscerosomatic index significantly increase in juvenile Jian carp (*Cyprinus carpio* var. Jian), and high fat content inhibits protein and carbohydrate utilization.

In mammals, the liver is the primary organ for lipid metabolism, with key proteins in hepatocytes participating in this process. Triglycerides enter the lymphatic circulation and are hydrolyzed into glycerol and fatty acids by adipose triglyceride lipase, hormone-sensitive lipase (HSL), and monoglyceride lipase. Residual chylomicrons, having lost most triglycerides, bind to hepatic receptors such as low-density lipoprotein (LDL) receptors, LDL receptor-related proteins, or scavenger receptor class B type I (SR-BI), entering hepatocytes through endocytosis. In the liver, integral transmembrane proteins such as the fatty acid transport protein (FATP) family (FATP-1~6) facilitate the transport of long-chain and very long-chain fatty acids from plasma into cells. On the cell membrane, fatty acids bind to fatty acid binding proteins (FABPs) and are transported through the cytosol for degradation or storage. Therefore, SR-BI, FATP4, and FABP10 are closely associated with fatty acid transport across cell membranes or within cells. Additionally, studies on invertebrates have revealed that Toll-like receptor (TLR) pathways play important roles in innate immunity, with feed nutrition closely related to gene expression in this pathway.

Oriental river prawn (*Macrobrachium nipponense*), also known as freshwater prawn, is one of the major freshwater aquaculture species in Southeast Asia and China. Currently, no studies have reported on dietary carbohydrate to lipid ratios for this species. Given the important physiological roles of carbohydrate to lipid ratios in aquatic animals, this experiment used oriental river prawn as a model to investigate the effects of dietary carbohydrate to lipid ratio on growth performance, antioxidant capacity, hepatopancreas metabolic

enzyme activities, fatty acid transport, and TLR pathway-related gene expression, aiming to determine the optimal dietary carbohydrate to lipid ratio and provide theoretical data for developing efficient and environmentally friendly formulated feeds for oriental river prawn.

Materials and Methods

1.1 Experimental Diet Preparation

Casein and fish meal served as protein sources, pregelatinized corn starch as carbohydrate source, and fish oil and soybean oil as lipid sources to formulate five isonitrogenous (crude protein content: 39%) experimental diets with carbohydrate to lipid ratios of 1.12 (CL1), 2.00 (CL2), 3.56 (CL3), 7.10 (CL4), and 24.12 (CL5). The carbohydrate to lipid ratios were determined by maintaining constant gross energy; the maximum pregelatinized corn starch addition level was obtained when lipid source addition was zero, while the maximum lipid source addition level was obtained at minimum pregelatinized corn starch addition. Lipid and pregelatinized corn starch additions were then proportionally decreased or increased. During diet preparation, all ingredients were ground to pass through a 60-mesh sieve, accurately weighed, and thoroughly mixed using a stepwise expansion method for micro-components such as vitamin and mineral premixes. The mixed fish oil and soybean oil with lecithin were then added and kneaded evenly, followed by water addition and mixing to form a dough. The dough was pelleted into 1.5 mm diameter particles using a small feed pelletizer, dried at 40 °C until moisture content reached approximately 10%, sealed, and stored at -20 °C.

Dietary crude protein content was determined by the Kjeldahl method (Kjeltec 2200 Kjeldahl Analyzer, FOSS, Denmark), crude lipid content by Soxhlet extraction (Soxtec™ 2043 Fat Analyzer, FOSS, Denmark), crude ash content by muffle furnace incineration at 550 °C for 14 h, moisture content by drying at 105 °C for 24 h to constant weight, gross energy by oxygen bomb calorimetry (WELL 9000, Shanghai), and crude fiber content by cellulose analyzer (ANKOM A200i, USA). Nitrogen-free extract content was calculated using the formula: nitrogen-free extract = 100 - (moisture + crude ash + crude protein + crude lipid + crude fiber). Carbohydrate to lipid ratio was calculated as the ratio of nitrogen-free extract content to crude lipid content according to methods described in references.

1.2 Experimental Animals and Management

Oriental river prawns were purchased from Huzhou Bangda Ecological Agriculture Co., Ltd. After one week of acclimation, healthy prawns with uniform body weight [average body weight: (0.104±0.003) g] were selected for the experiment. The prawns were randomly divided into 5 groups with 5 replicates per group and 50 prawns per replicate, and randomly placed in 300 L aquaria. Mesh sheets were placed in each aquarium as shelters to reduce cannibalism. The

experiment was conducted from July to September 2017. Each morning, feces and uneaten feed were siphoned and approximately one-third of the water was replaced. Aerated tap water was used with the following water quality parameters: temperature 25–29 °C, pH 7.6–8.1, dissolved oxygen concentration >6.5 mg/L, and total ammonia nitrogen concentration <0.01 mg/L. Prawns were fed twice daily (morning and afternoon) at 4%–5% of body weight. The feeding trial lasted 8 weeks.

1.3 Sample Collection

At the end of the feeding trial, after 24 h of starvation, prawns were weighed and survival numbers were recorded. Hepatopancreas tissues were dissected from the cephalothorax of surviving prawns in each group and stored at -80 °C for subsequent analysis of metabolic enzyme activities, antioxidant indices, fatty acid transport, and immune-related gene expression.

1.4 Growth Performance

Growth-related indices were calculated as follows: - Survival rate (SR, %) = 100 × (number of surviving prawns at experiment end) / (number of prawns at experiment start) - Weight gain rate (WGR, %) = 100 × (mean final body weight - mean initial body weight) / (mean initial body weight) - Specific growth rate (SGR, %/d) = 100 × (ln mean final body weight - ln mean initial body weight) / experimental days

1.5 Hepatopancreas Metabolic Enzyme Activities and Antioxidant Indices

Approximately 0.500 g of hepatopancreas was weighed and homogenized in ice-cold 0.86% physiological saline at a 1:9 mass-to-volume ratio to prepare 10% homogenate, which was centrifuged at 3,500 r/min for 15 min. The supernatant was collected and diluted to appropriate concentrations for various assays. Protein content in the supernatant was determined by the Coomassie brilliant blue method. Hepatopancreas glycogen, malondialdehyde (MDA) content, and activities of hexokinase (HK), pyruvate kinase (PK), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (T-AOC) were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer' s instructions.

1.6 Fatty Acid Transport and TLRs Pathway-Related Gene Expression Analysis

Total RNA was extracted from hepatopancreas using an RNA extraction kit (Beijing Aidlab Biotechnologies Co., Ltd.) according to the manufacturer' s instructions. RNA integrity was assessed by electrophoresis, and concentration and purity were determined by nucleic acid-protein analyzer. RNA was reverse

transcribed to cDNA using a reverse transcription kit (TaKaRa, Japan), and cDNA was stored at -20 °C for quantitative real-time PCR (qRT-PCR) analysis.

Primers for qRT-PCR of fatty acid transport-related genes (SR-BI, FABP10, FATP4) and TLR pathway-related genes (myeloid differentiation primary response protein 88 [MyD88], Toll-like receptor 3 [TLR3], mitogen-activated protein kinase kinase kinase 7 [MAP3K7], tumor necrosis factor receptor associated factor 6 [TRAF6], interleukin 1 receptor associated kinase 4 [IRAK4], and mitogen-activated protein kinase 14 [MAPK14]) were designed using online Primer 3 software (Table 2). The qRT-PCR reaction volume was 20 L, containing 10 L 2×SYBR Green Premix Ex Taq (TaKaRa, Japan), 0.2 L each of 10 mol/L forward and reverse primers, 2 L template, and 7.6 L ddH O. The qRT-PCR conditions were: 95 °C for 30 s; 40 cycles of 94 °C for 15 s, 58 °C for 20 s, and 72 °C for 20 s. After PCR, melting curves were generated by increasing temperature from 60 °C to 95 °C at 5 °C/5 s to verify amplification specificity. -actin (forward primer: 5' -GTGCCCATCTACGAGGGTTA-3' , reverse primer: 5' -CGTCAGGGAGCTCGTAAGAC-3') was used as the reference gene for normalization of Ct values. Gene expression levels were analyzed using the 2^(-ΔΔCt) method with CL5 group as the calibrator.

1.8 Statistical Analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA was performed using SPSS 19.0. When significant differences were detected, Tukey' s multiple comparison test was applied. The significance level was set at $P < 0.05$.

Results

2.1 Effects of Dietary Carbohydrate to Lipid Ratio on Growth Performance of Oriental River Prawn

As shown in Table 3 , no significant differences were observed in weight gain rate and specific growth rate when carbohydrate to lipid ratio increased from 1.12 (CL1) to 7.10 (CL4) ($P>0.05$). However, when carbohydrate to lipid ratio further increased to 24.12 (CL5), weight gain rate and specific growth rate were significantly reduced ($P<0.05$). No significant differences were observed in survival rate among all groups ($P>0.05$).

2.2 Effects of Dietary Carbohydrate to Lipid Ratio on Glycogen Content and Glucose Metabolic Enzyme Activities in Hepatopancreas

As shown in Table 4 , hepatopancreas glycogen content in CL5 group was significantly higher than that in CL4 group ($P<0.05$), while no significant differences were observed among CL1, CL2, CL3, and CL4 groups ($P>0.05$). The highest HK and PK activities in hepatopancreas were observed in CL4 group; HK activity in CL4 group was significantly higher than that in CL1 group ($P<0.05$),

while PK activity was significantly higher than all other groups (P<0.05).

2.3 Effects of Dietary Carbohydrate to Lipid Ratio on Antioxidant Indices in Hepatopancreas

As shown in Table 5 , no significant differences were observed in hepatopancreas MDA content, T-AOC, and CAT activity among groups fed diets with different carbohydrate to lipid ratios (P>0.05). However, hepatopancreas SOD activity in CL1 and CL2 groups was significantly higher than that in CL4 and CL5 groups (P<0.05).

2.4 Effects of Dietary Carbohydrate to Lipid Ratio on Expression of Fatty Acid Transport-Related Genes in Hepatopancreas

Figure 1 [Figure 1: see original paper] shows the effects of dietary carbohydrate to lipid ratio on expression of fatty acid transport-related genes in hepatopancreas. The expression level of SR-BI gene was highest in CL1 group and significantly higher than all other groups (P<0.05), with no significant differences among the remaining groups (P>0.05). Similarly, FATP4 gene expression level was highest in CL1 group and significantly higher than all other groups (P<0.05). FABP10 gene expression level in CL4 group was significantly higher than that in CL1, CL2, and CL3 groups (P<0.05), while no significant differences were observed among CL1, CL2, and CL3 groups (P>0.05) or between CL4 and CL5 groups (P>0.05).

[Figure 1: see original paper]

2.5 Effects of Dietary Carbohydrate to Lipid Ratio on Expression of TLRs Pathway-Related Genes in Hepatopancreas

Figure 2 [Figure 2: see original paper] shows the effects of dietary carbohydrate to lipid ratio on expression of immune-related genes in hepatopancreas. Expression of TLR pathway-related genes (TLR3, MyD88, IRAK4, TRAF6, MAP3K7, and MAPK14) was regulated by dietary carbohydrate to lipid ratio. Specifically, expression levels of TLR3, MyD88, IRAK4, TRAF6, MAP3K7, and MAPK14 genes in CL3 group were significantly higher than those in all other groups (P<0.05).

[Figure 2: see original paper]

Discussion

This study found that dietary carbohydrate to lipid ratios ranging from 1.12 to 7.10 did not significantly affect growth performance of oriental river prawn, but excessively high carbohydrate to lipid ratio (24.12) inhibited growth, indicating limited carbohydrate utilization capacity in this species. This is consistent with previous findings that high dietary carbohydrate levels are detrimental to growth of oriental river prawn and similar to results in redclaw crayfish and

fish species where excessively high carbohydrate to lipid ratios impair growth. Interestingly, high dietary lipid combined with low carbohydrate (carbohydrate to lipid ratio of 1.12) did not reduce or improve growth performance, though whether it causes metabolic disorders requires further investigation. Crustacean lipid requirements are influenced by various nutrients, making precise requirements difficult to determine. Studies have shown that dietary lipid levels of 4.2%–13.76% satisfy normal growth of swimming crab, and high-lipid diets did not significantly affect growth performance of tilapia, suggesting that both crustaceans and fish possess regulatory mechanisms when dietary lipid levels are high.

PK and HK are key enzymes in the glycolytic pathway. This study demonstrated that carbohydrate to lipid ratio significantly affected glucose metabolic enzyme activities in hepatopancreas. When dietary carbohydrate to lipid ratio increased from 1.12 to 7.10, HK and PK activities significantly increased, indicating that oriental river prawn can oxidize dietary carbohydrates at certain levels for energy utilization. This is similar to findings in Jian carp where PK and HK activities significantly increased when carbohydrate to lipid ratio rose from 2.3 to 7.7, and in redclaw crayfish where HK and PK activities increased when carbohydrate to lipid ratio rose from 1.33 to 10.75. However, when carbohydrate to lipid ratio further increased, PK activity significantly decreased, possibly because high carbohydrate content (35%) could not stimulate PK activity, indirectly indicating limited carbohydrate utilization capacity in oriental river prawn. Regarding glycogen content, high carbohydrate levels significantly increased hepatopancreas glycogen content, suggesting that unusable carbohydrates were stored as hepatic glycogen.

The hepatopancreas is the primary metabolic center for free radicals in crustaceans. Since cell membranes contain phospholipid bilayers that are vulnerable to free radical attack, lipid peroxidation occurs readily. MDA, a product of lipid peroxidation, is commonly used to measure endogenous oxidative stress. To reduce oxidative stress and maintain free radical homeostasis, organisms have evolved various antioxidant defense mechanisms, including specialized antioxidant enzymes such as SOD and CAT. This study found that dietary carbohydrate to lipid ratio did not significantly affect hepatopancreas MDA content, indicating that different dietary carbohydrate to lipid ratios did not induce significant oxidative stress. However, numerous studies have shown that high-lipid or high-carbohydrate diets cause oxidative stress, possibly due to species-specific tolerance to carbohydrates or lipids. Regarding hepatopancreas SOD activity, a decreasing trend was observed with increasing carbohydrate to lipid ratio, suggesting that reactive oxygen species (ROS) levels decreased as dietary lipid content decreased.

To further investigate whether dietary carbohydrate to lipid ratio affected lipid metabolism and immune function, we examined expression of fatty acid transport- and TLR pathway-related genes in hepatopancreas. The CD36 scavenger receptor superfamily plays crucial roles in lipid metabolism regulation and

innate immunity. SR-BI, a member of this family, participates in cellular lipid metabolism, maintains intracellular cholesterol homeostasis, and is important for membrane lipid expression and apoptosis. SR-BI in oriental river prawn contains a CD36 domain and may have similar fatty acid transport functions as CD36, with its expression regulated by dietary lipid sources. This study found that low dietary carbohydrate to lipid ratio not only promoted SR-BI gene expression but also enhanced long-chain fatty acid transport protein FATP4 gene expression. This differs somewhat from tilapia studies showing that carbohydrate to lipid ratio did not significantly affect FATP5 gene expression in liver, possibly due to differences in fatty acid transport protein types or species-specific responses. However, expression of intracellular liver-type fatty acid binding protein FABP10 gene significantly increased with increasing carbohydrate to lipid ratio (i.e., low lipid), suggesting that intracellular fatty acid transport may not correlate with membrane fatty acid transport. Tilapia studies also showed that low dietary lipid increased FABP4 gene expression compared to high- and medium-lipid groups, indicating enhanced intracellular fatty acid transport. The regulatory mechanisms of dietary carbohydrate to lipid ratio on expression of these fatty acid transport-related genes remain unclear and require further investigation.

Compared with vertebrates' sophisticated immune systems, invertebrates primarily rely on innate immunity for pathogen defense. TLR pathways play important roles in innate immunity. TLRs can activate inflammatory factor expression through MyD88-dependent and MyD88-independent pathways. Studies have shown that nutrients such as fatty acids can regulate expression of TLR and MyD88 genes. This study found that carbohydrate to lipid ratio of 3.56 (CL3) significantly upregulated TLR3 and MyD88 gene expression. TRAF6 acts as a molecular bridge connecting upstream TLR, MyD88, and IRAK genes with downstream nuclear factor- B (NF- B) and mitogen-activated protein kinase (MAPK) signaling pathways. In this study, these upstream and downstream molecules, including IRAK4, TRAF6, MAP3K7, and MAPK14 genes, were also regulated by dietary carbohydrate to lipid ratio, showing similar expression patterns to TLR3 and MyD88 genes. Although limited reference data exist on carbohydrate to lipid ratio effects on TLR pathway gene expression, studies have shown that appropriate carbohydrate to lipid ratios can improve immunity. Therefore, upregulated TLR pathway gene expression may be associated with enhanced non-specific immune response in oriental river prawn. TLR pathway genes are also involved in inflammatory responses that can alleviate tissue damage caused by pathogens or oxidative stress. In this study, increased carbohydrate to lipid ratio decreased hepatopancreas SOD activity, indicating reduced ROS production. Although TLR pathway gene expression was significantly lower in CL4 and CL5 groups, expression patterns in CL1, CL2, and CL3 groups were inconsistent with SOD activity changes, suggesting that altered TLR pathway gene expression may not be entirely caused by oxidative stress. Lipopolysaccharide (LPS) can activate TLR pathways, but repeated LPS stimulation induces tolerance and cross-tolerance, where cells show hyporespon-

siveness to subsequent LPS stimulation and broadly reduced reactivity to TLR ligands. In this study, the reduced expression of hepatopancreas TLR pathway genes after 8 weeks of feeding high-lipid (CL1 and CL2) or high-carbohydrate (CL4 and CL5) diets may involve similar tolerance mechanisms, though intermediate sampling in future studies is needed for verification. Additionally, TLR pathway gene expression characteristics are tissue-specific; TLR22 and MyD88 genes show opposite expression patterns in grass carp liver and kidney. This study only analyzed hepatopancreas TLR pathway gene expression, and whether other tissues show similar or opposite regulatory patterns requires further investigation.

Conclusion

1. Oriental river prawn exhibits considerable adaptability to dietary carbohydrate to lipid ratio, with no significant changes in weight gain rate at ratios of 1.12–7.10. However, excessively high carbohydrate to lipid ratio (24.12) inhibits growth, reduces key glycolytic enzyme activities in hepatopancreas, and increases hepatic glycogen accumulation.
2. Dietary carbohydrate to lipid ratio can regulate expression of fatty acid transport-related genes (SR-BI, FATP4, and FABP10) and TLR pathway-related genes (TLR3, MyD88, IRAK4, TRAF6, MAP3K7, and MAPK14) in hepatopancreas.

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