Effects of Dietary Carbohydrate To Lipid Ratios On Growth Performance, Body Composition, Serum Biochemical Indexes, Lipid Metabolism And Gene Expression of Central Appetite Regulating Factors In Chinese Perch (*Siniperca Chuatsi*)

Di Peng

Huazhong Agricultural University: Huazhong Agriculture University

Xu-Fang Liang (✉ xufang_liang@hotmail.com)

Huazhong Agricultural University: Huazhong Agriculture University

Farui Chai

Huazhong Agricultural University: Huazhong Agriculture University

Hexiong Feng

Huazhong Agricultural University: Huazhong Agriculture University

Jiao Li

Huazhong Agricultural University: Huazhong Agriculture University

Shulin Tang

Huazhong Agricultural University: Huazhong Agriculture University

Ke Lu

Huazhong Agricultural University: Huazhong Agriculture University

Qiwei Zhang

Huazhong Agricultural University: Huazhong Agriculture University

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Abstract

An 8-week feeding trial was conducted to evaluate the effects of dietary carbohydrate to lipid (CHO: L) ratios on growth performance, body composition, serum biochemical indexes, lipid metabolism and gene expression of central appetite regulating factors in Chinese perch (*Siniperca chuatsi*) (mean initial weight: 12.86 ± 0.10 g). Five isonitrogenous and isoenergetic diets (fish meal, casein as main protein sources) were formulated to contain different graded CHO:L ratio diets ranging from 0.12, 0.86, 1.71, 3.29 and 7.19. Each diet was assigned to triplicate groups of 18 experimental fish for 8 weeks. Our results revealed that final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER) increased with dietary CHO:L ratio from 0.12 to 1.71, and then decreased with further increases in dietary CHO:L ratio. A two-slope broken-line regression analysis based on WGR showed that the optimal dietary CHO: L level for maximum growth performance of fish was 1.60. Crude lipid and crude protein content in the liver and glycogen concentration in the muscle and liver were significantly influenced by the dietary CHO:L ratios ($P < 0.05$). The lowest crude lipid content in the liver was observed in fish fed the diet with a CHO:L ratio of 1.71 ($P < 0.05$). Dietary CHO:L ratios significantly induced the Glu contents of serum ($P < 0.05$). The relative expression levels of genes involved in lipid metabolism, such as *srebp1* and *fas* in the liver showed a trend of first decreased and then increased with the increase of dietary CHO:L ratios levels. Appropriate CHO:L ratio in the diet can effectively reduce the accumulation of liver fat. We observed in fish fed the 1.71 CHO:L ratio diet showed higher feed intake, up-regulated mRNA expression of neuropeptide Y (NPY) and agouti gene-related protein (AGRP), down-regulated mRNA expression of cocaine-and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) significantly as compared to control group. Thus, these results provide the theoretical basis for feed formulation to determine the appropriate CHO:L ratio requirement of Chinese perch.

Introduction

Over the past three decades, the growth rate of global aquaculture production is significantly higher than that of fishing, and it is the fastest growing industry in the global agricultural sector. Fish meal has become an important means to meet the demand for protein of aquatic animals and land animals. However, the demand for human consumption of fish is also increasing, resulting in high costs and fluctuations in fishmeal supply (FAO 2018, Montoya-Camacho et al 2019). To address this issue, in addition to constantly seeking high-quality protein sources that can replace fish meal, researchers are also improving feed formulation to save protein and improve feed protein efficiency (Turchin et al 2009). Lipids and carbohydrates are one of the most important nutrients for organisms, and carbohydrates are a relatively inexpensive source of energy (Gao et al 2018). Previous results demonstrate that appropriate lipid and carbohydrate levels in diets can provide energy to organisms and have the effect of saving protein (Hongyan et al 2019, Misra et al 2005, Sehrish et al 2020, Zhao et al 2020). Lipids provide essential fatty acids for the growth and development of fish, are the main structure of cell membranes, and facilitate the transport and absorption of fat-soluble vitamins in the body (Xue et al 2020, Zhaokun et al 2020). Carbohydrates also have good adhesion and expandability, which can improve the stability of

feed in water (Shi-Mei et al 2018). Excessive lipid will cause a series of problems such as physiological metabolic disorders, poor growth and fatty liver (Luet al 2014, Zhou et al 2020). Fish are also born with diabetes, especially carnivorous fish (Wilson 1994). Excessive carbohydrate level will lead to excessive metabolic burden, long-term hyperglycemia, excessive accumulation of liver glycogen, decreased immune function and other obstacles (Kamalam et al 2016, Shi-Mei et al 2018). In fish, glucose is closely related to lipid metabolism. Dihydroxyacetone phosphate produced in the process of glycolysis is then converted into glycerol needed for fat synthesis. Acetyl CoA generated from glucose oxidative decomposition is also a precursor for fatty acid synthesis, which can promote the generation of fat in the body (Liet al 2020). Dietary lipid can also be converted to glucose by gluconeogenesis. (Honorato et al 2010). Fish appetite is co-regulated by the central system and the peripheral system, through the signal transduction between various appetite regulating factors (including appetite promoting factors and appetite suppressant factors), the "appetite regulating network" of fish is formed (Het al 2005, Yokobori et al 2012). Food intake is closely related to appetite, and there is evidence in humans, other mammals and fish that the intake of a high-fat diet can suppress appetite and reduce sensitivity to the present food (bet al 2017, Liet al 2016, Ortinau et al 2014, Rasmussen et al 2015). In bony fish, fed high carbohydrate diet or injecting glucose intraperitoneally caused rainbow trout (*Oncorhynchus mykiss*) (Figueiredo-Silva et al 2013), sea bass (*Dicentrarchus labrax*) (Castro et al 2015b), and Japanese flounder (*Paralichthys olivaceus*) (Liet al 2018) decreased appetite and food intake. In recent years, researchers have made a high number of studies on the effects of dietary CHO:L ratio on growth performance, feed utilization and antioxidant capacity of fish (Gao et al 2010, Liet al 2012b, Zhou et al 2016). Nevertheless, there are few studies on the effects of different dietary CHO:L ratios on lipid metabolism and appetite of fish.

The Chinese perch, which is mainly distributed in China, Russia, Korea, Vietnam and other regions, is one of the unique freshwater aquaculture fish with great economic value in China (Liet al 2020a). It is a typical carnivorous fish with fierce feeding habits. It hunts live prey fish from its mouth-opening and could stably accept artificial compound feed after domestication (Lian et al 2010, Lian et al 2001). Currently, the protein content in the commercial feed of Chinese perch is extremely high, so changing the CHO:L ratios in the diet to improve the utilization rate of protein of Chinese perch, reduce the content of fish meal, and thus reduce the feed cost is of great significance for intensive farming. In fact, there are relatively few studies on nutritional requirements of Chinese perch. This study aims to explore the effects of different dietary CHO:L ratios on growth performance, body composition, liver lipid metabolism, liver histomorphology and appetite of Chinese perch. The results of this study may provide a basis for the development of high efficiency compound feed in the future.

Materials And Methods

All animal care and experimental procedures in the present study were approved by Huazhong Agricultural University and conducted in accordance with the Guidelines for Experimental Animals (Ethical code: HZAUF-2020-0004).

2.1. Experimental diets

The formulation and proximate composition of the experimental diets are provided in Table 1. Five isonitrogenous and isoenergetic experimental diets were formulated to contain different levels of CHO:L ratio (0.12, 0.86, 1.71, 3.29 and 7.19). Experimental diets were compounded with fish meal and casein as main protein sources, fish oil and soybean oil as main lipid sources, and dextrin as a carbohydrate source. All dry ingredients were thoroughly mixed in a mixer before the addition of oil and 40 % water. Then, the mixture was then pelleted (4 mm diameter) by a laboratory pelleting machine (HR 2330 model, PHILIPS, Suzhou, China). The soft pellets were placed in hermetic bags were stored in a freezer at – 20°C until used.

Table 1
Formulation and proximate chemical compositions of the tested diets.

Dietary C/L levels (%)					
Ingredients (%)	D1	D2	D3	D4	D5
Fish meal	46	46	46	46	46
Casein	18	18	18	18	18
Dextrin	4	10	16	22	28
Fish oil	5.5	4.2	2.9	1.6	0.3
Soybean oil	5.5	4.2	2.9	1.6	0.3
Vitamin supplement ¹	1	1	1	1	1
Mineral supplement ²	2	2	2	2	2
Sodium alginate	2	2	2	2	2
Cellulose	14	10.6	7.2	3.8	0.4
Proximate Compositions					
Dry matter (% DM)	93.68	93.60	93.53	93.23	92.92
Crude protein (% DM)	48.64	47.82	48.57	48.35	48.13
Crude lipid (% DM)	14.62	11.49	8.97	6.53	3.85
Crude ash (% DM)	12.79	12.30	12.51	12.19	12.74
Energy (MJ·kg ⁻¹) ³	17.55	17.53	17.65	17.68	17.64
Crude fiber (% DM)	15.91	12.08	8.10	4.69	0.53
Nitrogen-free extract (% DM)	1.72	9.91	15.38	21.47	27.67
Carbohydrate: lipid (CHO:L)	0.12	0.86	1.71	3.29	7.19
1. Vitamin premix: choline, 1000 mg/kg; inositol, 600 mg/kg; vitamin A, 40 mg/kg; vitamin D3, 60 µg/kg; vitamin E, 200 mg/kg; vitamin K3, 10 mg/kg; vitamin B1 (thiamin), 15 mg/kg; vitamin B2 (riboflavin), 25 mg/kg; vitamin B6, 20 mg/kg; pantothenic acid calcium, 50 mg/kg; niacinamide, 200 mg/kg; biotin, 3.2 mg/kg; vitamin B12, 0.1 mg/kg; folic acid, 10 mg/kg; ascorbic acid, 210 mg/kg.					
2. Mineral premix: CaHPO4, 15 g/kg; KCl, 3 g/kg; MgSO4, 0.8 g/kg; NaCl, 1.5 g/kg; CuSO4·5H2O, 10 mg/kg; FeSO4, 150 mg/kg; ZnSO4, 80 mg/kg; MnSO4, 13 mg/kg; Na2SeO3, 0.7 mg/kg; KI, 1.1 mg/kg; Na2MoO4, 0.14 mg/kg; CoSO4, 0.02 mg/kg; KF 0.26 mg/kg.					
3. Gross energy was calculated using energy equivalents 18.81, 35.57, and 14.59 kJ/g for protein, lipid and digestible carbohydrate, respectively					

2.2. Experimental fish and feeding management

The experiment was conducted in an indoor aquarium system at the Chinese Perch Research Center, Huazhong Agricultural University, Wuhan, China. About 500 Chinese perch were purchased from Wuhongshan Breeding Base (Chibi, China). Before the feeding experiment, Chinese perch were trained to accept the artificial diet according to the method of Liang et al. (2001). After acclimatization, 270 fish (mean initial weight: 12.86 ± 0.10 g) with uniform size, accepts artificial diet well and health were randomly assigned to 15 tanks with 18 fish per tank. Fish were hand-fed twice (08:00 and 19:00) fed till satiation daily. The amount of feed being consumed by fish in each tank was recorded daily. Uneaten feed was collected by siphoning after 15-min feeding and then oven-dried at $60\text{ }^{\circ}\text{C}$ to calculate feed intake. During the feeding period, the water temperature, pH, and dissolved oxygen (DO) level were $19 - 23\text{ }^{\circ}\text{C}$, $7.1 - 7.5$, and $> 5\text{ mg/L}$, respectively. Ordinary photoperiod applied throughout the experiment.

2.3. Sample collection and analysis

After 8 weeks of feeding trial, all fish were starved for 24 h, and then counted individually and weighed. Six fish were randomly selected from each replicate were anaesthetized with 3-aminobenzoic acid ethyl ester methane sulphonate (MS-222, 50 mg/L water). The blood was obtained from caudal vein of fish by syringe using a 1-mL syringe and pooled into a sterile centrifuge tube to clot overnight at $4\text{ }^{\circ}\text{C}$, then centrifuged at $4000\times g$ at $4\text{ }^{\circ}\text{C}$ for 20 min to separate the serum and stored at $-80\text{ }^{\circ}\text{C}$ until used for analysis. Then these fish were dissected on ice to obtain brain, muscle, liver, intestine, mesentery and visceral adipose, quickly frozen in liquid nitrogen and cryopreserved at $-80\text{ }^{\circ}\text{C}$ for subsequent analysis. Liver tissues from three fishes from each group were collected and immediately fixed by 4 % paraformaldehyde for histological evaluation. Another 3 fish were randomly chosen from each tank for whole-body composition.

The data were analysed for Weight Gain Rate (WGR), Specific Growth Rates (SGR), Hepatosomatic index (HSI), Viscera index (VSI), Mesentery fat index (MFI), Survival Ratio (SR), Feed intake (FI) and Food Conversion Ratio (FCR) using the following formula:

Weight gain rate (WGR, %) = $100 \times (\text{final weight} - \text{initial weight}) / \text{initial weight}$

Specific growth rate (SGR, %/d) = $100 \times (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days}$

Hepatosomatic index (HSI, %) = $100 \times \text{hepatopancreas weight} / \text{body weight}$

Viscera index (VSI, %) = $\text{viscera weight} \times 100 / \text{body weight}$

Mesentery fat index (MFI, %) = $\text{the weight of mesenteric fat} / \text{body weight}$

Survival rate (SR, %) = $100 \times \text{final number of fish} / \text{initial number of fish}$

Food intake (FI, $\text{g fish}^{-1} \text{ days}^{-1}$) = $\text{total amount of the feed consumed} / \text{number of fish} / \text{days}$

Feed conversion ratio (FCR, %) = $\text{amount of feed given} / \text{weight gain}$

Crude protein, crude lipid, ash and moisture of diets, whole body, muscle, and liver were measured according to standard Association of Official Agricultural Chemists methods (International and Technology 1995). Crude protein content was determined by measuring nitrogen ($N \times 6.25$) levels using the Kjeldahl method following acid digestion with an auto Kjeldahl System (Kjelflex K-360; BUCHI Labortechnik AG, Flawil, Switzerland). Moisture content was measured by freeze-drying samples for 48 h in a vacuum freeze dryer (Christ Beta 2–4 LD plus LT, Marin Christ Corporation, Osterode, Germany). Ash level was examined through incineration at 550 °C for 24 h in a muffle furnace.

Serum total protein (TP), albumin (ALB), glucose (GLU), total cholesterol (TCHO), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) contents, along with aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined using an automatic biochemical analyser (CHEMIX-800, Sysmex Corporation, Kobe, Japan) with commercial diagnostic reagent kits (Sysmex Wuxi Co. Ltd., Wuxi, China).

Total RNA was extracted by RNAiso Plus reagent (Takara, Dalian, China) manually, and the purity and quantity of RNA were determined by agarose gel electrophoresis, protein, and nucleic acid analyser. Then 1 µg of total RNA was used for reverse transcription with HiScript® II Reverse Transcriptase (Code no. R301–01/02; Vazyme, China) in a 20 µL reaction volume to synthesize cDNA. Specific primers for the candidate genes used for qPCR were designed based on previous published research paper of our laboratory (Table 2). Rpl13a gene was used as an endogenous reference to normalize the template amount. The total volume of the qRT-PCR reaction system was 20 µL, including 10 µL of SYBR Green dye (Code no. Q311–02; Vazyme, China), 0.4 µL of PCR forward/reverse primers (10 µM), 1 µL of cDNA template and 8.2 µL RNase-free H₂O. The qRT-PCR amplification programme was 95°C for 1 min, followed by 40 cycles consisting of 95°C for 10 s and 57°C for 30 s and a melt curve step (from 95°C, gradually reducing 0.5°C/s to 57°C, with data acquisition every 6 s). The amplification efficiencies of control and target genes were approximately equal and ranged from 96.3–104.9%. Gene expression levels were quantified relative to the expression of rpl13a using the optimized comparative Ct ($2^{-\Delta\Delta Ct}$) value method. All amplifications were performed in triplicate for each RNA sample.

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

Gene name	Primer	Sequence 5'-3'	Annealing temp (°C)
<i>acca</i>	<i>acca</i> -F	TATGCCCACTTACCCAAATGC	58
	<i>acca</i> -R	TGCCACCATACCAATCTCGTT	
<i>fas</i>	<i>fas</i> -F	ATGGAAATCACCCCTGTAATCTT	57
	<i>fas</i> -R	CTTATCTGACTACGGAATGAATCG	
<i>cpt1</i>	<i>cpt1</i> -F	ATGGTGTATTGGCTGGAGTCT	57.5
	<i>cpt1</i> -R	CTGTGTGGTAGGTTTTTCCTTGAT	
<i>srebp1</i>	<i>Srebp1</i> -F	CTCCCTCCTTTCTGTCGGCTC	58
	<i>Srebp1</i> -R	TCATTTGCTGGCAGTCGTGG	
<i>npy</i>	<i>npy</i> -F	GTTGAAGGAAAGCACAGACA	58
	<i>npy</i> -R	GCTCATAGAGGTAAAAGGGG	
<i>agrp</i>	<i>AgRP</i> -F	GAGCCAAGCGAAGACCAGA	60
	<i>AgRP</i> -R	GCAGCACGGCAAATGAGAG	
<i>pomc</i>	<i>pomc</i> -F	GGCTGAAGATGGTGTGTCTATG	58
	<i>pomc</i> -R	ACATGCAGAGGTGAATACAGTC	
<i>cart</i>	<i>cart</i> -F	CGAACCTAACCAGTGAGAAG	56
	<i>cart</i> -R	GGGACAGTCGCACATCTT	
<i>rpl13a</i>	<i>rpl13a</i> -F	TATCCCCCACCCTATGACA	59
	<i>rpl13a</i> -R	ACGCCCAAGGAGAGCGAACT	

2.4. Statistical Analysis

All data were analysed by the Statistical Software SPSS for Windows (v. 26). Data were described as means $\pm$ SEM of triplicate group. The data were subjected to independence sample *t* test to check the differences between control and experimental groups. Differences were considered significant at $P \leq 0.05$.

Results

3.1. Growth performance and feed utilization

As show in Table 3, SR did not show significant difference among fish fed the different dietary treatments ($P>0.05$). Fish fed D3 had the highest FBW, WGR and SGR ($P<0.05$). PER in fish fed D3 was significantly higher than that in fish fed other diet ($P<0.05$). FI was significantly affected by the dietary CHO:L ratios ($P<0.05$), fish fed D3 was significantly higher than D1, D2, D4 groups. HSI and VSI in the D1 was significantly higher than that in the other groups ($P<0.05$). The IPR of D4, D5 groups were significantly higher than that of D1, D2, D3 groups ($P<0.05$). FCR exhibited an opposite trend as observed in WGR, with the lowest level in fish fed D3 group ($P<0.05$).

Table 3
Growth parameters of Chinese perch fed the experimental diets.

Dietary C/L levels (%)					
	D1	D2	D3	D4	D5
IBW(g)	14.29 ± 0.27	13.88 ± 0.28	13.47 ± 0.65	14.22 ± 0.87	14.19 ± 0.06
FBW(g)	21.49 ± 0.57 ^a	31.22 ± 1.42 ^b	39.59 ± 2.08 ^c	30.51 ± 1.50 ^b	33.99 ± 1.24 ^b
WGR (%)	85.70 ± 6.94 ^a	124.82 ± 8.63 ^{bc}	193.86 ± 6.37 ^d	114.86 ± 4.79 ^b	139.66 ± 9.54 ^c
SGR (%/d)	1.10 ± 0.07 ^a	1.44 ± 0.07 ^b	1.92 ± 0.04 ^c	1.36 ± 0.04 ^b	1.56 ± 0.07 ^b
PER	0.81 ± 0.02 ^a	1.17 ± 0.10 ^b	1.51 ± 0.06 ^c	1.14 ± 0.08 ^b	1.24 ± 0.09 ^b
FI(g)	0.56 ± 0.03 ^{ab}	0.55 ± 0.01 ^{ab}	0.64 ± 0.01 ^c	0.53 ± 0.02 ^a	0.59 ± 0.01 ^{bc}
HSI (%)	2.71 ± 0.28 ^b	1.79 ± 0.08 ^a	1.73 ± 0.07 ^a	1.84 ± 0.10 ^a	1.66 ± 0.07 ^a
VSI (%)	11.56 ± 0.58 ^c	8.58 ± 0.42 ^a	10.01 ± 0.40 ^b	8.35 ± 0.28 ^a	8.34 ± 0.27 ^a
IPR (%)	0.82 ± 0.14 ^b	0.98 ± 0.12 ^b	0.90 ± 0.07 ^b	0.51 ± 0.05 ^a	0.31 ± 0.05 ^a
SR (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
FCR	2.57 ± 0.07 ^c	1.80 ± 0.16 ^b	1.39 ± 0.05 ^a	1.85 ± 0.14 ^b	1.81 ± 0.15 ^b
Values are presented as the means ± SEM (n = 6). Values within a column followed by different superscript letters differ significantly ($P<0.05$).					

Based on broken-line regression analysis of WGR, the dietary CHO:L ratios for optimum growth of Chinese perch was 1.60, corresponding to 15.38% of nitrogen-free extract and 8.97% of crude lipid respectively, belongs to the D3 group (Fig. 1).

3.2. Proximate composition and glycogen content in tissues

Effects of dietary CHO:L ratios on whole body, proximate composition and glycogen content of Chinese perch are listed in Table 4. The lipid content of whole body trended downward as dietary CHO:L ratios increased. There were no significant differences in moisture, crude protein and crude lipid contents of the

muscle among all treatments ($P > 0.05$). Dietary CHO:L ratios significantly affected the contents of crude protein and lipid in liver of Chinese perch ($P < 0.05$). Fish fed D3 had lower crude lipid content in liver than other groups, and significantly lower than D1 and D4 groups ($P < 0.05$).

Table 4

The body composition of whole fish and muscle of Chinese perch fed the experimental diets.

Dietary C/L levels (%)					
	D1	D2	D3	D4	D5
Whole body					
Moisture (%)	74.23 ± 0.18	73.98 ± 0.68	74.69 ± 0.47	74.98 ± 0.43	75.16 ± 0.58
Crude protein (%)	17.19 ± 0.32	17.50 ± 0.37	17.18 ± 0.69	16.69 ± 0.65	16.35 ± 0.76
Crude lipid (%)	15.83 ± 0.59 ^{ab}	17.54 ± 2.41 ^b	15.56 ± 1.84 ^{ab}	13.76 ± 0.75 ^{ab}	11.36 ± 1.93 ^a
Muscle					
Moisture (%)	77.95 ± 0.77	77.85 ± 0.46	79.32 ± 0.17	78.51 ± 0.83	79.22 ± 0.25
Crude protein (%)	19.44 ± 0.45	20.01 ± 0.38	18.40 ± 0.72	19.68 ± 0.58	18.55 ± 1.03
Crude lipid (%)	5.09 ± 0.99	4.17 ± 0.63	4.20 ± 1.01	2.95 ± 0.39	3.75 ± 0.06
Muscle glycogen/(mg/g)	3.12 ± 0.10 ^a	3.11 ± 0.11 ^a	3.42 ± 0.22 ^{ab}	3.58 ± 0.08 ^{bc}	3.81 ± 0.04 ^c
Liver					
Moisture (%)	74.41 ± 3.35	69.49 ± 0.71	70.53 ± 0.45	71.31 ± 0.97	73.69 ± 1.40
Crude protein (%)	11.68 ± 1.48 ^a	15.03 ± 1.31 ^b	15.02 ± 0.43 ^b	14.01 ± 0.64 ^{ab}	16.03 ± 0.39 ^b
Crude lipid (%)	14.79 ± 1.10 ^b	12.75 ± 1.93 ^{ab}	9.24 ± 0.44 ^a	14.04 ± 1.33 ^b	11.81 ± 1.33 ^{ab}
Liver glycogen/(mg/g)	26.90 ± 1.17 ^a	39.55 ± 2.63 ^b	51.49 ± 2.15 ^c	55.48 ± 6.62 ^c	54.00 ± 2.89 ^c
Values are presented as the means ± SEM (n = 6). Values within a column followed by different superscript letters differ significantly ($P < 0.05$).					

The muscle glycogen and liver glycogen had an increase trend with the increasing CHO:L ratios level. The highest muscle glycogen content was observed in D5 group, and significantly higher than D1, D2 and D3 groups. There was no significant difference in liver glycogen content among D3, D4 and D5 groups ($P > 0.05$).

3.3. Serum biochemical indices

Dietary CHO:L ratios significantly induced the contents of GLU (Table 5). TP and ALB were significantly influenced by dietary CHO:L ratios, and it were the lowest in D3 among all groups, and was significantly lower than that in D1, D2 and D4 ($P < 0.05$). The highest concentration of TCHO and TG were found in fish fed a diet with the lowest CHO:L ratio. The activities of AST and ALT both increased with dietary CHO:L ratios, showing a trend of first decreased and then increased, and reached the lowest in D3 group ($P < 0.05$). As dietary CHO:L ratio increased, serum HDLC content was significantly decreased ($P < 0.05$). On the contrary, LDLC was not significantly influenced by dietary CHO:L ratio ($P > 0.05$).

Table 5
Serum biochemical indexes of Chinese perch fed the experimental diets.

Dietary C/L levels (%)					
	D1	D2	D3	D4	D5
TP(g/L)	41.42 ± 1.40 ^c	40.70 ± 1.52 ^c	31.97 ± 1.52 ^a	38.24 ± 1.66 ^{bc}	35.63 ± 1.48 ^{ab}
ALB(g/L)	11.63 ± 0.40 ^{bc}	11.86 ± 0.4 ^c	9.80 ± 0.43 ^a	11.86 ± 0.46 ^c	10.50 ± 0.31 ^{ab}
TCHO(mmol/L)	8.18 ± 0.22 ^b	7.84 ± 0.25 ^b	6.42 ± 0.23 ^a	6.96 ± 0.22 ^a	6.59 ± 0.19 ^a
TG(mmol/L)	8.77 ± 0.37 ^c	7.70 ± 0.23 ^b	7.42 ± 0.29 ^{ab}	6.89 ± 0.37 ^{ab}	6.70 ± 0.21 ^a
GLU(mmol/L)	8.75 ± 0.41 ^a	9.34 ± 0.43 ^a	11.90 ± 0.55 ^b	11.43 ± 0.74 ^b	11.77 ± 0.46 ^b
AST(U/L)	108.19 ± 13.33 ^c	65.45 ± 12.43 ^b	28.53 ± 8.07 ^a	68.84 ± 14.99 ^b	94.52 ± 12.40 ^c
ALT(U/L)	121.01 ± 8.47 ^d	79.11 ± 5.49 ^c	30.08 ± 3.29 ^a	72.16 ± 10.41 ^{bc}	56.28 ± 6.01 ^b
HDLC(mmol/L)	0.98 ± 0.04 ^b	0.87 ± 0.04 ^b	0.67 ± 0.04 ^a	0.68 ± 0.04 ^a	0.59 ± 0.03 ^a
LDLC(mmol/L)	1.10 ± 0.20	1.26 ± 0.26	1.04 ± 0.34	1.05 ± 0.22	1.05 ± 0.25
Values are presented as the means ± SEM (n = 6). Values within a column followed by different superscript letters differ significantly ($P < 0.05$)					

3.4. Relative expression of lipid metabolism-related genes in liver

The expressions of lipid metabolism-related genes in Chinese perch fed different dietary CHO:L ratios levels are presented in Fig. 2. The expression of *srebp1* and *fas* in the liver showed a trend of first decreased and then increased with the increase of dietary CHO:L ratios. And the expressions of *srebp1* and *fas* in the liver were the lowest in the D3 group, which was significantly lower than the other groups ($P < 0.05$). Compared with the D1 group, the expression of *acca* in the liver of the other groups was

significantly reduced, and there was no significant difference ($P>0.05$). The expression level of *cpt-1* in the liver of D3 and D5 group was significantly higher than that of D1 group ($P<0.05$) (Fig. 3).

3.5. Relative expression of appetite-related genes in hypothalamus

Concerning appetite regulation-related genes are presented in Fig. 4 and Fig. 5. Compared with the D1 group, the expression of *npv* in the D2 and D3 groups was significantly increased ($P<0.05$). The expression of *agrp* increased with the increase in the level of dietary CHO:L ratios, showing a trend of first increased and then decreased. The expression levels of *npv* and *agrp* were the highest in the D3 group and were significantly higher than the other groups ($P<0.05$). With the increase of dietary CHO:L ratios, the expression of *cart* decreased significantly compared with the D1 group ($P<0.05$). The expression of *pomc* in the D2 and D3 groups were significantly lower than the other groups ($P<0.05$).

3.6. Histology analyses of liver section and determination of hepatocyte inflammation

Figure 6 shows the oil red O staining and H&E staining of Chinese perch liver tissue fed with different levels of dietary CHO:L ratios diet. The Oil-red O staining confirmed that the number of red dots (lipid droplets) has exhibited no obvious difference among D2 and D4 group, but it increased sharply in D1 and D5 group. The D3 group had the least number of lipid droplets (Fig. 6A). H&E staining confirmed that except for the D3 group, the liver cells of the other treatment groups showed more different numbers of small vacuoles, and the hepatocyte nuclei were squeezed to the edge (Fig. 6B). This means that the liver cells have different degrees of pathological reactions.

Discussion

In the present study, we provided direct evidence that dietary CHO:L ratio of 1.71 advanced the growth performance of Chinese perch. At this point, the WGR, SGR and PER of the Chinese perch reach the maximum. When the dietary CHO:L ratio was 0.12, the WGR, SGR and PER were all lower than those of the other groups. This indicates that a relatively low- or high- CHO:L ratio diet not only depressed Chinese perch growth but also caused their poor feed utilization. Protein utilization efficiency can be improved by adjusting the ratio of carbohydrates to lipids in the feed. (Jobling 2012). Similar results were found in other carnivorous fish. Appropriate CHO:L ratio was helpful for fish to exert synergistic effect in the utilization of carbohydrate and lipid, improve the utilization rate of feed, and thus promote growth. Such as large yellow croaker (*Larimichthys crocea*) (Zhou et al 2016), juvenile hybrid grouper (*epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) (Gao et al 2018), juvenile cobia (*Rachycentron canadum*) (Zhao et al 2020) and juvenile black seabream (*Acanthopagrus schlegelii*) (Sehrish et al 2020). 0.12 CHO:L group showed poor growth, indicating that high-lipid/low-carbohydrate group was not conducive to its growth, and its VSI and HSI were higher than those in other groups, indicating that higher dietary lipid level would affect lipid deposition in viscera and liver. (Zhou et al 2020). Polyline regression analysis based on WGR revealed that the dietary CHO: L requirements of Chinese perch could be satisfied if the CHO:L ratio reached 1.60. Growth, feed efficiency and protein utilization ratio suggest that carbohydrates are a better digestible energy source for Chinese perch compared with lipids and can save protein.

Although the carbohydrate utilization capacity of carnivorous fish is weak, however, the carbohydrate utilization level of fish is a result of interactions with the physical state of dietary starch, molecular complexity, glucose tolerance, environment, temperature, and other nutrient elements in the feed (Braugeet al 1995, Hemre et al 2015, Liet al 2019b).

In this study, dietary CHO:L ratio had no significant effect on muscle composition but had significant effect on crude lipid content of whole fish and liver. The lipid content of whole fish generally decreases and the lipid content of liver a decreasing trend first and then increasing trend in response to increasing dietary CHO: L ratio in feed. It indicates that the crude lipid content of whole body is positively correlated with the dietary lipid level, and excessive lipid will be deposited in fish body. However, fish have a poor ability to utilize carbohydrate. When the high-carbohydrate/low-lipid, fish may decompose part of the body lipid for energy supply, such as the lipid in muscle. Similar results were found in large yellow croaker (*Larimichthys crocea*)(Liet al 2019c) and golden pompano (*Trachinotus ovatus*)(Donget al 2018). In the liver, both high lipid levels and high carbohydrate levels contribute to fat deposition. These results were similar to those previously reported in largemouth bass (*Micropterus salmoides*) (Zhou et al 2020) and blunt snout bream (*Megalobrama amblycephala*) (Wanget al 2017).

The blood index parameters could indicate the physiological and health status of fish and could change dynamically with the nutritional status of fish (Fazio 2019). In this study, TG, TCHO, and HDLC in serum were significantly decreased with the increase of CHO: L ratio in the diet, indicating that endogenous lipid transport in fish was also more active under the high lipid diet. Metabolites rich in TG and CHO in liver were transported to other abdominal organs along with blood circulation, resulting in the increase of VSI. The serum Glu content was significantly increased with the dietary CHO: L ratio increasing. Liver glycogen significantly increased in the dietary CHO: L of 0.12 to 1.71 and then leveled off. The results show that the Chinese perch could use a certain level of carbohydrate(<15.38%) in feed and store it in liver in the form of liver glycogen. However, the Chinese perch still cannot make good use of carbohydrate. When the dietary carbohydrate level is higher than 15.38%, the serum Glu content increases continuously, leading to hyperglycemia and partial glycogen storage in the muscle. Similar results have been observed in some carnivorous fish(Mingchun et al 2011, Zhou et al 2016). Serum total protein content is a major indicator of physiological health of fish (Alexander et al 2011). The albumin and globulin contained in it are generally considered to play an important role in the innate immune response of fish (Liet al 2012a, Wiegertjes et al 1996). ALT and AST are considered sensitive indicators of normal tissue function and are often used to determine whether the liver has been damaged(Xing et al 2020). In this study, when dietary CHO:L ratio was 1.71, TP, ALB, ALT and AST were significantly lower than those in other groups. These results indicate that high dietary fat or carbohydrate levels will affect the health of the fish, increase the burden on the liver, and thus activate the non-specific immunity of the fish. In some previous studies, such as European sea bass (*Dicentrarchus labrax* L.) (Sitjà-Bobadilla and Pérez-Sánchez 1999), blunt snout bream(*Megalobrama amblycephala*) (Zhou et al 2013) have been reported.

Lipid deposition in the liver is a comprehensive result of lipid uptake, transport, decomposition, and synthesis in hepatocytes(Cet al 2012, Xing et al 2020). *srebp-1* is a transcription factor that can activate

target genes of its downstream fatty acid synthesis(Kuipers et al 2011). *fas* and *acca* are important enzymes in fat synthesis, which are involved in the synthesis of fatty acids (Castro et al 2016). In this study, *srebp-1* and *fas* showed a trend of decreasing first and then increasing with the increase of dietary CHO:L ratio, indicating that fatty acid synthesis in liver was more active in the diet of high-lipid/low-carbohydrate or high-carbohydrate/low-lipid. In addition, the increase of dietary carbohydrate level may also be the reason for the increase of liver *srebp-1*, which can also mediate the conversion of excess carbohydrates to fatty acids (Ferré and Foufelle 2010, Miriamet al 2008), fatty acids enter the liver and are esterified into triglycerides, which are stored in lipid droplets on the one hand and enter liver cells, On the other hand, as TG-rich metabolites, they are secreted into blood for circulation(Yuan et al 2016). However, serum TCHO, TG and LDLC levels did not increase in the high-carbohydrate/low-lipid group (D4 and D5), indicating that fatty acids may mainly exist in liver lipid droplets, which may cause harm to the health of fish. And *cpt-1* is the key gene of fatty acid β oxidation. The *cpt-1* in liver of Chinese perch was significantly up-regulated when the dietary CHO:L ratio is 1.71, indicating that the β -oxidation of fatty acids is more active under this nutritional state. At this time, liver fat was significantly lower than that of the other groups, indicating that liver of Chinese perch can effectively alleviate liver fat accumulation by reducing fatty acid synthesis and increasing fatty acid β oxidation when feeding appropriate CHO:L ratio diet.

Feed is the main cost in aquaculture, so the feed intake is considerable important to the aquaculture of economic fish, and the feed intake is controlled by the central and peripheral appetite network system(Liet al 2019a, Marta and L. 2017). In previous studies, fish's appetite was influenced by the nutritional content of the feed and the proportion of different ingredients added. By Catarina Basto-Silva studies have shown that different dietary protein to energy ratio affects the feeding intake and appetite regulation of gilthead seabream(*Sparus aurata*)(Catarina et al 2021). The central system, especially the hypothalamus, can sense the nutritional state of the body, regulate food intake and metabolism through different neural circuits, and maintain the energy homeostasis of the body(Clémente and J 2010). These circuits mainly include *npv/agrp*, *pomc/cart* neurons, and central and peripheral endocrine factors that respond to circulating glucose, fatty acid, or amino acid levels, respectively(Blouet and Schwartz 2009, Efeyan et al 2015, Mobbset al 2005). In previous studies based on Chinese perch in our laboratory, central nervous system *npv/agrp* was generally used as an appetite promoting factor, while *pomc/cart* was used as an appetite suppressant factor (Liu et al 2020b). In this study, with the increase of dietary CHO:L ratio, the food intake of Chinese perch in D3 group was significantly higher than that in other treatment groups. The expression of *npv/agrp* neurons in the hypothalamus of Chinese perch was firstly increased and then decreased in response to increasing CHO:L ratio in feed. However, the expression level of inhibitory appetite factor *cart* in D1 group was significantly higher than that in other treatment groups, and the expression level of *pomc* in D2 and D3 groups was significantly downregulated. These results indicated that high lipid or high carbohydrate diet could inhibit the expression of appetite promoting genes *npv* and *agr* to some extent and promote the expression of appetite suppressing genes *cart* and *pomc*. In previous studies, high carbohydrate diets can induce European sea bass (*Dicentrarchus labrax*) (Castro et al 2015a), rainbow trout(*Oncorhynchus mykiss*) (Cristina et al 2016), tilapia(*Oreochromis mossambicus*)

(Otero-Rodino et al 2016), and Siberian sturgeon (*Acipenser baeri*) (Gong et al 2015) reduced food intake. High lipid diets also decrease food intake of fish (Yong-Jun et al 2018). Therefore, appropriate CHO:L ratio feed can promote appetite, which has a positive effect on increasing food intake to avoid anorexia in the process of breeding.

Conclusion

In conclusion, through broken line regression analysis, the optimal carbohydrate-to-lipid ratio in the diet of Chinese perch is recommended to be 1.71. The ratio of sugar to lipid in the diet affected the growth and feeding of Chinese perch, the antioxidant indexes in serum and the accumulation of glycogen in body. In addition, the appropriate carbohydrate-to-lipid ratio in the diet up-regulated or down-regulated the expression of lipid metabolism genes in the liver and reduced the lipid deposition in the liver. Appropriate CHO:L ratio affects the transcription level of hypothalamus appetite gene, thus increasing food intake. The results of this study can provide an important reference for the optimization of compound feed for Chinese perch.

Declarations

Acknowledgments

D.P., and X-F L. designed the experiments and draft the manuscript. D.P., F-R C., and H-X F. performed the experiments. All authors read and approved the final manuscript.

Data availability statement

All data are available from the corresponding author by request.

Ethics declarations

All experiments and animal-handling procedures were approved by the Ethics Committee of the Institute of Laboratory Animal Centre, Huazhong Agriculture University (Ethical code: HZAUF-2020-0004).

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of Interest

All authors of this research paper have directly participated in the planning, execution, or analysis of this study. No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

References

1. Alexander C, Sahu NP, Pal AK et al (2011) Haemato-immunological and stress responses of Labeo rohita (Hamilton) fingerlings: effect of rearing temperature and dietary gelatinized carbohydrate[J]. Journal of Animal Physiology Animal Nutrition 95(5):653–663
2. Frédéric Tantot a b, L P A B CS, C A R M B et al., The effect of high-fat diet consumption on appetitive instrumental behavior in rats[J]. Appetite, 2017, 108: 203–211
3. Blouet C, Schwartz GJ (2010) Hypothalamic nutrient sensing in the control of energy homeostasis. Behav Brain Res 209(1):1–12 PubMed, 209(1) :1–12.
4. Brauge C, Corraze G, Médale F, Effects of dietary levels of carbohydrate and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout, Oncorhynchus mykiss, reared in freshwater or in seawater [J]. Comparative Biochemistry & Physiology Part A Physiology, 1995, 111(1):pp 117–124
5. C SG (2012) S.O. V, and H.W. S, Fish oil – how does it reduce plasma triglycerides? [J]. BBA - Molecular Cell Biology of Lipids 1821(5):843–851
6. Castro C, Corraze G, Basto A et al (2016) Dietary Lipid and Carbohydrate Interactions: Implications on Lipid and Glucose Absorption, Transport in Gilthead Sea Bream (*Sparus aurata*) Juveniles[J]. Lipids 51(6):1–13
7. Castro C, Corraze G, Pérez-Jiménez A et al (2015) Dietary carbohydrate and lipid source affect cholesterol metabolism of European sea bass (*Dicentrarchus labrax*) juveniles[J]. Br J Nutr 114(08):1143–1156
8. Catarina B-S, Paula E, Aires O-T et al., Dietary protein source and protein/carbohydrate ratio affects appetite regulation-related genes expression in gilthead seabream (*Sparus aurata*) - ScienceDirect[J]. Aquaculture, 2020, 533
9. Clémence B (2010) and S.G. J, Hypothalamic nutrient sensing in the control of energy homeostasis [J]. Behav Brain Res 209(1):1–12
10. Cristina O-R, Cristina V, Rosa Á-O et al., In vitro evidence supports the presence of glucokinase-independent glucosensing mechanisms in hypothalamus and hindbrain of rainbow trout[J]. Journal

- of Experimental Biology, 2016: jeb.137737
11. Dong LF, Tong T, Zhang Q et al., Effects of dietary carbohydrate to lipid ratio on growth, feed utilization, body composition and digestive enzyme activities of golden pompano (*Trachinotus ovatus*) [J]. John Wiley & Sons, Ltd (10.1111). 2018. 24(1)
 12. Efeyan A, Comb WC, Sabatini DM (2015) Nutrient-sensing mechanisms, and pathways[J]. Nature 517(7534):302
 13. FAO, The State of World Fisheries and Aquaculture 2018:Meeting the Sustainable Development Goals (2018) United Nations. 2018
 14. Fazio F, Fish hematology analysis as an important tool of aquaculture: A review [J]. Aquaculture, 2019. 500
 15. Ferré P, Foufelle F, Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c.[J]. Diabetes Obesity & Metabolism, 12
 16. Figueiredo-Silva AC, Saravanan S, Schrama JW et al (2013) A comparative study of the metabolic response in rainbow trout and Nile tilapia to changes in dietary macronutrient composition[J]. Br J Nutr 109(05):816–826
 17. Gao W, Liu YJ, Tian LX et al (2010) Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*) [J]. Aquac Nutr 16(3):327–333
 18. Gao Y, Luo Y, Li X et al., Effects of dietary carbohydrate/lipid ratios on growth, feed utilization, hematology parameters, and intestinal digestive enzyme activities of juvenile hybrid grouper (Brown-Marbled Grouper *Epinephelus fuscoguttatus* × *Giant Grouper E. lanceolatus*) [J]. North American Journal of Aquaculture, 2018. 80(4)
 19. Gong G, Xue M, Wang J et al., The regulation of gluconeogenesis in the Siberian sturgeon (*Acipenser baeri*) affected later in life by a short-term high-glucose programming during early life[J]. Aquaculture, 2015. 436
 20. H V, F CL, U. S, et al., Neuropeptides and the control of food intake in fish[J]. General and comparative endocrinology, 2005. 142(1–2)
 21. Hemre GI, Mommsen TP, Nutrition KJA (2015) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes[J]. Aquac Nutr 8(3):175–194
 22. Hongyan L, Wenjie X, Junyan J et al., Effects of Dietary Carbohydrate and Lipid Concentrations on Growth Performance, Feed Utilization, Glucose, and Lipid Metabolism in Two Strains of Gibel Carp[J]. Frontiers in veterinary science, 2019. 6
 23. Honorato CA, Almeida LC, Nunes C et al (2010) Effects of processing on physical characteristics of diets with distinct levels of carbohydrates and lipids: the outcomes on the growth of pacu (*Piaractus mesopotamicus*) [J]. Aquac Nutr 16(1):91–99
 24. International, AJ.T.i.FS, Technology, Official methods of analysis of AOAC International, 16th edition. Volume 2 (1995) 6(11): p. 382–382

25. Jobling MJAI, National Research Council (NRC): Nutrient requirements of fish and shrimp. 2012. 20(3): p. 601–602
26. Kamalam BS, Medale F, Panserat S (2017) Utilisation of dietary carbohydrates in farmed fishes: New insights on influencing factors, biological limitations and future strategies [J]. *Aquaculture* 467(1):3–27
27. Kuipers RS, Luxwolda MF, Sango WS et al (2011) Postpartum changes in maternal and infant erythrocyte fatty acids are likely to be driven by restoring insulin sensitivity and DHA status[J]. *Med Hypotheses* 76(6):794–801
28. Li A, Yuan X, Liang XF et al (2016) Adaptations of lipid metabolism and food intake in response to low and high fat diets in juvenile grass carp (*Ctenopharyngodon idellus*) [J]. *Aquaculture* 457:43–49
29. Li L, Fang J, Liang XF et al., Effect of feeding stimulants on growth performance, feed intake and appetite regulation of mandarin fish, *Siniperca chuatsi*[J]. *Aquaculture Research*, 2019(8)
30. Li S, Sang C, Wang A et al., Effects of dietary carbohydrate sources on growth performance, glycogen accumulation, insulin signaling pathway and hepatic glucose metabolism in largemouth bass, *Micropterus salmoides* [J]. *Aquaculture (Amsterdam, Netherlands)*, 513:734391–734391
31. Li S, Yin J, Zhang H et al., Effects of dietary carbohydrate and lipid levels on growth performance, feed utilization, body composition and non-specific immunity of large yellow croaker (*Larimichthys crocea*) [J]. *Aquaculture Nutrition*, 2019, 25(5)
32. Li X-F, Liu W-B, Lu K-L et al., Dietary carbohydrate/lipid ratios affect stress, oxidative status and non-specific immune responses of fingerling blunt snout bream, *Megalobrama amblycephala*[J]. *Fish & Shellfish Immunology*, 2012, 33(2):pp 316–323
33. Li Z, Wen-Bin L (2020) B.P. B., et al., Utilization of raw and gelatinized starch by blunt snout bream *Megalobrama amblycephala* as evidenced by the glycolipid metabolism, glucose tolerance and mitochondrial function[J]. *Aquaculture* 529:735603
34. Liang X, Liu J, Huang B et al (2010) The role of sense organs in the feeding behaviour of Chinese perch[J]. *J Fish Biol* 52(5):1058–1067
35. Liang X, Oku H, Ogata H et al (2015) Weaning Chinese perch *Siniperca chuatsi* (Basilewsky) onto artificial diets based upon its specific sensory modality in feeding[J]. *Aquac Res* 32(s1):76–82
36. Liu D, Guo B, Han D et al., Comparatively study on the insulin-regulated glucose homeostasis through brain-gut peptides in Japanese flounder *Paralichthys olivaceus* after intraperitoneal and oral administration of glucose [J]. *General and Comparative Endocrinology*, 2018:9–20
37. Liu L, Fang J, Liang XF et al., Nucleotide promotes feed intake and protein utilization via regulating the gene expression of feeding and nitrogen metabolism in juvenile Chinese perch (*Siniperca chuatsi*)[J]. *Aquaculture Nutrition*. 2020b. 26(5)
38. Lu KL, Xu WN, Wang LN et al (2014) Hepatic β -Oxidation and Regulation of Carnitine Palmitoyltransferase (CPT) I in Blunt Snout Bream *Megalobrama amblycephala* Fed a High Fat Diet [J]. *PLOS ONE* 9(3):e93135-

39. Marta CS and S.J. L., Nutrient Sensing Systems in Fish: Impact on Food Intake Regulation and Energy Homeostasis [J]. *Frontiers in Neuroscience*, 2017, 10
40. Mingchun R, Qinghui et al., Effect of dietary carbohydrate level on growth performance, body composition, apparent digestibility coefficient and digestive enzyme activities of juvenile cobia, *Rachycentron canadum* L[J]. *Aquaculture Research*, 2011, 42(10)
41. Miriam E, Isidoro M, Marlon C et al (2007) Role of Sp1 and SREBP-1a in the insulin-mediated regulation of glucokinase transcription in the liver of gilthead sea bream (*Sparus aurata*) [J]. *Gen Comp Endocrinol* 155(2):359–367
42. Misra S, Sahu NP, Pal AK et al (2006) Pre- and post-challenge immuno-haematological changes in Labeo rohita juveniles fed gelatinised or non-gelatinised carbohydrate with n-3 PUFA [J]. *Fish Shellfish Immunol* 21(4):346–356
43. Mobbs CV, Isoda F, Makimura H et al., Impaired glucose signaling as a cause of obesity and the metabolic syndrome: The glucoadipostatic hypothesis[J]. *Physiology & Behavior*, 2005, 85(1):pp 3–23
44. Montoya-Camacho N, Marquez-Ríos E, Castillo-Yáñez FJ et al., Advances in the use of alternative protein sources for tilapia feeding[J]. *Reviews in Aquaculture*, 2019, 11(3)
45. Ortinau LC, Hoertel HA, Douglas SM et al (2014) Effects of high-protein vs. high- fat snacks on appetite control, satiety, and eating initiation in healthy women[J]. *Nutr J* 13(1):97
46. Otero-Rodino C, Velasco C, Alvarez-Otero R et al., In vitro evidence supports the presence of glucokinase-independent glucosensing mechanisms in hypothalamus and hindbrain of rainbow trout[J]. *Journal of Experimental Biology*, 2016: jeb.137737
47. Rasmussen O (2015) Rnsholdt, et al., Manipulation of end-product quality in rainbow trout with finishing diets[J]. *Aquac Nutr* 6(1):17–23
48. Sehrish T, Misbah I, Min J et al (2020) Effects of Dietary Carbohydrate to Lipid Ratios on Growth Performance, Muscle Fatty Acid Composition, and Intermediary Metabolism in Juvenile Black Seabream (*Acanthopagrus schlegelii*) [J]. *Front Physiol* 11:507
49. Shi-Mei L, Chao-Ming S, Ming-Ming M et al (2018) Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, *Micropterus salmoides*[J], 78. *Fish & Shellfish Immunology*, pp 121–126
50. Sitjà-Bobadilla A, PÉrez-SÁNchez J, SHORT COMMUNICATION Diet related changes in non-specific immune response of European sea bass (*Dicentrarchus labrax* L.) [J]. *Fish & Shellfish Immunology*, 1999, 9(8):pp 637–640
51. Turchini GM, Torstensen BE, Ng WK, Fish oil replacement in finfish nutrition [J]. John Wiley & Sons, Ltd (10.1111). 2009. 1(1)
52. Wang B-K, Liu W-B, Xu C et al., Dietary carbohydrate levels and lipid sources modulate the growth performance, fatty acid profiles and intermediary metabolism of blunt snout bream *Megalobrama amblycephala* in an interactive pattern [J]. *Aquaculture*, 2017. 481

53. Wiegertjes GF, Stet RM, Parmentier HK et al., Immunogenetics of disease resistance in fish: A comparative approach[J]. *Developmental & Comparative Immunology*, 1996, 20(6):pp 365–381
54. Wilson RPJA (1994) Utilization of dietary carbohydrate by fish - ScienceDirect. [J] *Aquaculture* 124(1–4):67–80
55. Xing L, Di P, Xiaoru C et al., Effects of dietary protein levels on growth, muscle composition, digestive enzymes activities, hemolymph biochemical indices and ovary development of pre-adult red swamp crayfish (*Procambarus clarkii*) [J]. *Aquaculture Reports*, 2020. 18
56. Xu H, Han T, Li X et al (2020) Effects of dietary lipid levels on survival, growth performance, and antioxidant ability of the early juvenile *Scylla paramamosain* [J]. *Aquaculture* 528:735559
57. Yokobori E, Azuma M, Nishiguchi R et al (2012) Neuropeptide Y Stimulates Food Intake in the Zebrafish, *Danio rerio*[J]. *J Neuroendocrinol* 24(5):766–773
58. Yong-Jun D, Guang-Zhen J, Xiang-Yang Y et al., High-fat-diet-induced inflammation depresses the appetite of blunt snout bream (*Megalobrama amblycephala*) through the transcriptional regulation of leptin/mammalian target of rapamycin[J]. *The British journal of nutrition*, 2018. 120(12)
59. Yuan X, Liang X-F, Liu L et al., Fat deposition pattern and mechanism in response to dietary lipid levels in grass carp, *Ctenopharyngodon idellus*[J]. *Fish Physiology and Biochemistry*. 2016. 42(6)
60. Zhao H, Cao J, Chen X et al (2020) Effects of dietary lipid-to-carbohydrate ratio on growth and carbohydrate metabolism in juvenile cobia(*Rachycentron canadum*) [J]. *Animal Nutrition* 6(01):80–84
61. Zhaokun D, Yongqiang L, Jinhua H et al., Dietary Lipid Supplementation Could Significantly Affect the Growth, Fatty Acid Profiles, and Expression of PPAR α , Leptin, and Adiponectin Genes in Juvenile Genetically Improved Farmed Tilapia [J]. *European Journal of Lipid Science and Technology*. 2020. 123(1)
62. Zhou C, Liu B, Ge X et al (2013) Effect of dietary carbohydrate on the growth performance, immune response, hepatic antioxidant abilities and heat shock protein 70 expression of Wuchang bream, *Megalobrama amblycephala*[J]. *J Appl Ichthyol* 29(6):1348–1356
63. Zhou P, Wang M, Xie F et al., Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker (*Larmichthys crocea*) [J] *Aquaculture*, 2016. 452
64. Zhou Y-L, Guo J-L, Tang R-J et al., High dietary lipid level alters the growth, hepatic metabolism enzyme, and anti-oxidative capacity in juvenile largemouth bass *Micropterus salmoides* [J]. *Fish Physiology and Biochemistry*, 2020. 46(1)

Figures

Figure 1

Broken-line analysis between the weight gain rate (WGR) of Chinese perch and dietary CHO: L level.

Figure 2

The mRNA expression levels of genes involved in lipid synthesis (srebp-1, sterol regulatory element-binding proteins-1; fas, fatty acid synthase; accα, acetyl-coA carboxylase alpha). Values are presented as the means ± SEM (n=6). Different lowercase letters above the bars indicate significant difference (P < 0.05).

Figure 3

The mRNA expression levels of genes involved in fatty acidβ-oxidation (cpt1, carnitine palmitoyl transferase). Values are presented as the means ± SEM (n=6). Different lowercase letters above the bars indicate significant difference (P < 0.05).

Figure 4

The mRNA expression levels of genes involved in appetite-promoting genes in the hypothalamus (*npy*, neuropeptide Y; *agrp*, agouti related neuropeptide). Values are presented as the means $\pm$ SEM (n=6). Different lowercase letters above the bars indicate significant difference (P < 0.05).

Figure 5

The mRNA expression levels of genes involved in appetite-inhibiting genes in the hypothalamus (*cart*, cocaine-and amphetamine-regulated transcript; *pomc*, proopiomelanocortin). Values are presented as the means $\pm$ SEM (n=6). Different lowercase letters above the bars indicate significant difference (P < 0.05).

Figure 6

Histology analyses of liver section and determination of hepatocyte inflammation. (A) Hepatic tissue section (40× magnification) of Oil Red O staining of Chinese perch fed with dietary different CHO: L level. (B) Hepatic tissue section (40× magnification) of hematoxylin and eosin staining of Chinese perch fed with dietary different CHO: L level. Lipid droplets appear red after staining Oil Red O, and the depth of color of the red stain and the amount of the lipid droplet were positively correlated with lipid content. The nuclei of hepatocyte appear blue and the vacuole present to be hyaline after staining hematoxylin and eosin, and the numbers of nuclei were negatively correlated with hepatic steatosis. Ld, lipid droplet; Nu, nuclei; Va, vacuole.