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Effects of dietary carbohydrate to lipid ratios on growth, biochemical indicators, lipid metabolism, and appetite in Chinese perch (*Siniperca chuatsi*)

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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. Our results revealed that final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), and 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 glucose concentration 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 ratio 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*), and down-regulated mRNA expression of cocaine- and amphetamine-regulated transcript (*cart*) and pro-opiomelanocorticoid (*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.

Keywords Carnivorous fish · CHO:L ratios · Feed intake · Growth performance · Liver lipid synthesis

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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 (Turchini 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(Li, et al 2019a, b, c, d; Misra et al 2005; Taj 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(Ding et al 2020; Xu et al 2020). Carbohydrates also have good adhesion and expandability, which can improve the stability of feed in water (Lin et al 2018). Excessive lipid will cause a series of problems such as physiological metabolic disorders, poor growth, and fatty liver (Lu et 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; Lin 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 (Zhang et 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 (Volkoff et al 2004; 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 (Li et al 2016; Ortinau et al 2014; Rasmussen et al 2000; Tantot et al. 2017). In bony fish, fed high carbohydrate diet or injecting glucose intraperitoneally caused rainbow trout (*Oncorhynchus mykiss*) (Figueiredo-Silva et al 2013), European sea bass (*Dicentrarchus labrax*) (Castro et al 2015), and Japanese flounder (*Paralichthys olivaceus*) (Liu et 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; Li et al 2012; 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 (*Siniperca chuatsi*), 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 (Liu et al 2020). 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 (Liang et al 2010, 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: HZAUFI-2020–0004).

Experimental diets

The formulation and proximate composition of the experimental diets are provided in Table 1. A dietary protein level of 48% was used for this study based on the findings of previous research in our laboratory (Wang et al. 2018); five isonitrogenous and isoenergetic experimental diets were formulated to contain different levels of CHO:L ratio: 0.12 (D1), 0.86 (D2), 1.71 (D3), 3.29 (D4), and 7.19 (D5). 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 (%)				
	D1	D2	D3	D4	D5
Ingredients (%)					
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					
Moisture	6.32	6.40	6.47	6.77	7.08
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)[3]	17.55	17.53	17.65	17.68	17.64
Crude fibre (% DM)	15.91	12.08	8.10	4.69	0.53
Nitrogen-free extract (% DM)[4]	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 D_3, 60 μg/kg; vitamin E, 200 mg/kg; vitamin K_3, 10 mg/kg; vitamin B_1 (thiamin), 15 mg/kg; vitamin B_2 (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 B_{12}, 0.1 mg/kg; folic acid, 10 mg/kg; ascorbic acid, 210 mg/kg

[2]Mineral premix: $CaHPO_4$, 15 g/kg; KCl, 3 g/kg; $MgSO_4$, 0.8 g/kg; NaCl, 1.5 g/kg; $CuSO_4{\cdot}5H_2O$, 10 mg/kg; $FeSO_4$, 150 mg/kg; $ZnSO_4$, 80 mg/kg; $MnSO_4$, 13 mg/kg; Na_2SeO_3, 0.7 mg/kg; KI, 1.1 mg/kg; Na_2MoO_4, 0.14 mg/kg; $CoSO_4$, 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

[4]Nitrogen-free extract content = 100 – moisture – crude protein – crude lipid – crude ash – crude fibre

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. (Liang et al 2001). After 2 weeks of acclimatization, 270 fish (mean initial weight: 12.86 ± 0.10 g) with uniform size accept artificial diet well and health and were randomly assigned to fifteen 400 l round fibreglass tanks (80 cm diameter × 80 cm height) with 18 fish per tank. Five feeds with different CHO:L ratio were weighed daily (3–5% total weight of per tank all fish) and hand-fed twice (08:00 and 19:00) to five different treatment groups till satiation. 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 °C to calculate feed intake. During the feeding period, approximately 25% of the water in each tank was replenished daily at 12:00 am. The water temperature, pH, and dissolved oxygen (DO) level were recorded daily, and their values were 19–23 °C, 7.1–7.5, and > 5 mg/l, respectively. Ordinary photoperiod applied throughout the experiment.

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 sulfonate (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 °C and then centrifuged at 4000 × g at 4 °C for 20 min to separate the serum and stored at – 80 °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 °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.

Growth parameters

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:

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

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

$$\text{Protein efficiency ratio(PER)} = (\text{total final body weight} - \text{total initial body weight})/\text{protein intake}$$

$$\text{Food intake(FI, g/fish/days)} = 100 \times \text{total amount of the dry feed consumed/number of fish/days of feeding trial}$$

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

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

$$\text{Mesentery fat index(MFI, \%)} = 100 \times \text{the wet weight of mesenteric fat/body wet weight}$$

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

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

Proximate composition

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 (Hirwitz and Latimer 1995). Crude protein content was determined by measuring nitrogen (N × 6.25) levels using the Kjeldahl method following acid digestion with an auto Kjeldahl System (Kjelflex K-360, BUCHI Labortechnik AG, Flawil, Switzerland). Crude lipid was determined by using the Soxhlet extraction procedure. 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). Crude ash content was examined through incineration at 550 °C for 24 h in a muffle furnace. The crude fibre was analysed by the fritted glass crucible method using an automatic analyser (FT12, Gerhardt, Germany).

Serum biochemical analysis

Serum total protein (TP), albumin (ALB), glucose (GLU), total cholesterol (TCHO), triglycerides (TG), high-density lipoprotein (HDLC), and low-density lipoprotein (LDLC) 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).

Analysis of genes expression

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;

Table 2 Primer sequences for the quantitative real-time PCR

Gene name	Primer	Sequence 5′-3′	Annealing temp (℃)
accα	accα-F	TATGCCCACTTACCCAAATGC	58
	accα-R	TGCCACCATACCAATCTCGTT	
fas	fas-F	ATGGAAATCACCCCTGTAATCTT	57
	fas-R	CTTATCTGACTACGGAATGAATCG	
cpt1	cpt1-F	ATGGTGTATTGGCTGGAGTCT	57.5
	cpt1-R	CTGTGTGGTAGGTTTTCCTTGAT	
srebp1	srebp1-F	CTCCCTCCTTTCTGTCGGCTC	58
	srebp1-R	TCATTTGCTGGCAGTCGTGG	
npy	npy-F	GTTGAAGGAAAGCACAGACA	58
	npy-R	GCTCATAGAGGTAAAAGGGG	
agrp	agrp-F	GAGCCAAGCGAAGACCAGA	60
	agrp-R	GCAGCACGGCAAATGAGAG	
pomc	pomc-F	GGCTGAAGATGGTGTGTCTATG	58
	pomc-R	ACATGCAGAGGTGAATACAGTC	
cart	cart-F	CGAACCTAACCAGTGAGAAG	56
	cart-R	GGGACAGTCGCACATCTT	
rpl13a	rpl13a-F	TATCCCCCCACCCTATGACA	59
	rpl13a-R	ACGCCCAAGGAGAGCGAACT	

Vazyme, China), 0.4 µl of PCR forward/re-verse primers (10 µM), 1 µl of cDNA template, and 8.2 µl RNase-free H_2O. The qRT-PCR amplification program 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 to 104.9%. Gene expression levels were quantified relative to the expression of rpl13a using the optimized comparative Ct (2-ΔΔCt) value method. All amplifications were performed in triplicate for each RNA sample.

Liver histopathological examination

According the standard methods at Wuhan Google Biological Technology Co., Ltd. (Wuhan, China), liver tissues from three fishes from each group were collected and immediately fixed by using 4% neutral buffered formaldehyde for 48 h. After dehydrated and imbedded into paraffin, a tissue section was cut into 5 µm for hematoxylin and eosin (H&E) staining. For the frozen section and Oil Red O staining, liver samples were immediately frozen with liquid nitrogen and stored at − 80 °C. Serial frozen sections were cut into 8 µm for Oil Red O staining.

Statistical analysis

The normality of the data was tested by a single sample *T* test. And homogeneity of all data was tested before one-way analysis of variance (ANOVA). The data were expressed as mean ± SEM (standard error of the mean) and were analysed by ANOVA. A multiple comparison test (Duncan's new multiple range test) was conducted to compare the significant differences among groups. Differences were considered significant at $P \leq 0.05$. The dietary optimum CHO:L ratio of Chinese perch was estimated by the broken-line regression. Statistical analysis was conducted by using the SPSS 26.0 (SPSS, Chicago, IL, USA).

Results

Growth performance and feed utilization

As shown 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 diets ($P < 0.05$). FI was significantly affected by the dietary CHO:L ratios ($P < 0.05$), fish fed D3 was significantly higher than D1, D2, and D4 groups. HSI and VSI in the D1 were significantly higher than that in the other groups ($P < 0.05$). The

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}
MFI (%)	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}

IBW initial body weight; *FBW* final body weight; *WGR* weight gain rate; *SGR* specific growth rate; *PER* protein efficiency ratio; *FI* food intake; *HSI* hepatosomatic index; *VSI* viscera index; *MFI* mesentery fat index; *SR* survival rate; *FCR* feed conversion ratio. Values are presented as the means ± SEM ($n = 6$). Values within a column followed by different superscript letters differ significantly ($P < 0.05$)

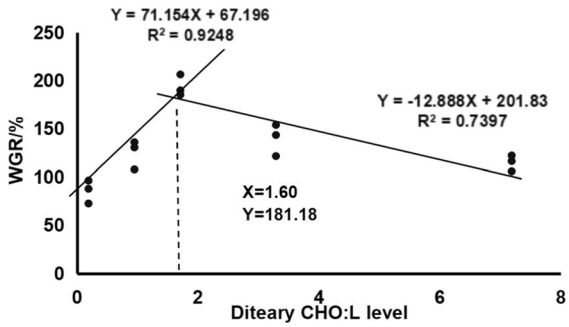

Fig. 1 Broken-line analysis between the weight gain rate (WGR) of Chinese perch and dietary CHO:L level

MFI of D4 and D5 groups were significantly lower than that of D1, D2, and D3 groups ($P<0.05$). FCR exhibited an opposite trend as observed in WGR, with the lowest level in fish fed D3 group ($P<0.05$).

Based on broken-line regression analysis of WGR, the dietary CHO:L ratio 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).

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 ($P<0.05$). Liver crude protein increased in D2, D3, and D5 groups compared with D1 group. Fish fed D3 had lower crude lipid content in liver than other groups and significantly lower than D1 and D4 groups ($P<0.05$).

The muscle glycogen and liver glycogen had an increase trend with the increasing CHO:L ratio 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 ($P>0.05$).

Serum biochemical indices

Dietary CHO:L ratios significantly induced the contents of GLU (Table 5). GLU in fish fed D1 and D2 were significantly lower than that in fish fed other diets ($P<0.05$). TP and ALB were significantly influenced by dietary CHO:L ratios, and it was the lowest in D3 among all groups and was significantly

Table 4 The body composition of whole fish and muscle of Chinese perch fed the experimental diets (% of wet weight)

	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 ($P<0.05$)

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

TP total protein; *ALB* albumin; *TCHO* total cholesterol; *TG* triglycerides; *GLU* glucose; *AST* aspartate transaminase; *ALT* alanine transaminase; *HDLC* high-density lipoprotein; *LDLC* low-density lipoprotein. Values are presented as the means ± SEM ($n = 6$). Values within a column followed by different superscript letters differ significantly ($P < 0.05$

lower than that in D1, D2, and D4 ($P < 0.05$). TCHO in fish fed D1 and D2 were significantly higher than other groups ($P < 0.05$). As dietary CHO:L ratio increased, serum TG content was significantly decreased ($P < 0.05$). The highest concentration of TG was 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 ($P > 0.05$).

Relative expression of lipid metabolism-related genes in the liver

The expressions of lipid metabolism-related genes in Chinese perch fed different dietary CHO:L ratio 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*

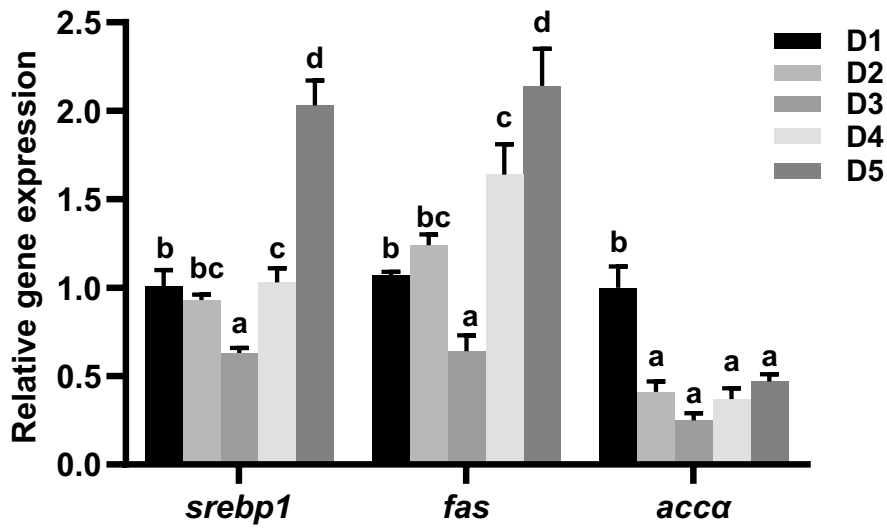

Fig. 2 The mRNA expression levels of genes involved in lipid synthesis (*srebp-1*, sterol regulatory element-binding proteins-1; *fas*, fatty acid synthase; *accα*, acetyl-coA carboxylase alpha). Values are presented as the means ± SEM ($n = 6$). Different lowercase letters above the bars indicate significant difference ($P < 0.05$)

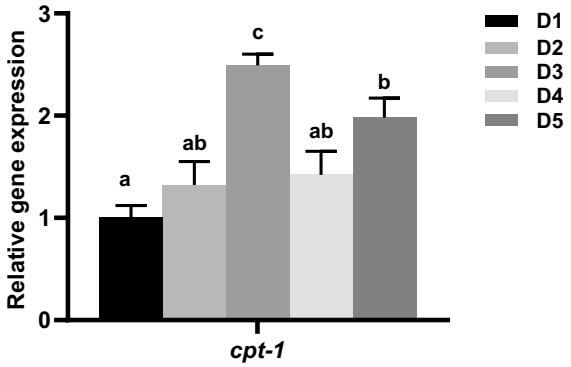

Fig. 3 The mRNA expression levels of genes involved in fatty acidβ-oxidation (*cpt1*, carnitine palmitoyl transferase). Values are presented as the means ± SEM ($n = 6$). Different lowercase letters above the bars indicate significant difference ($P < 0.05$)

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 *accα* 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).

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 *npy* 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 *npy* 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 ($P < 0.05$).

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.

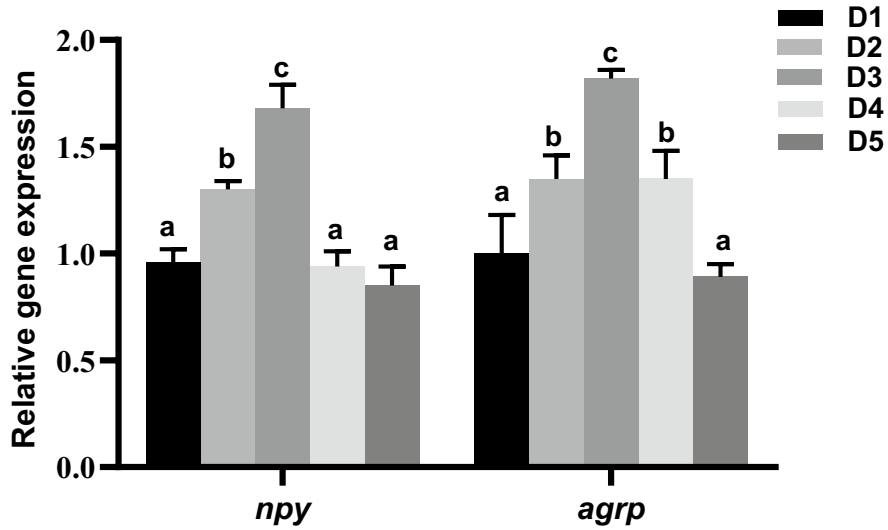

Fig. 4 The mRNA expression levels of genes involved in appetite-promoting genes in the hypothalamus (*npy*, *neuropeptide Y*; *agrp*, *agouti related neuropeptide*). Values are presented as the means ± SEM ($n = 6$). Different lowercase letters above the bars indicate significant difference ($P < 0.05$)

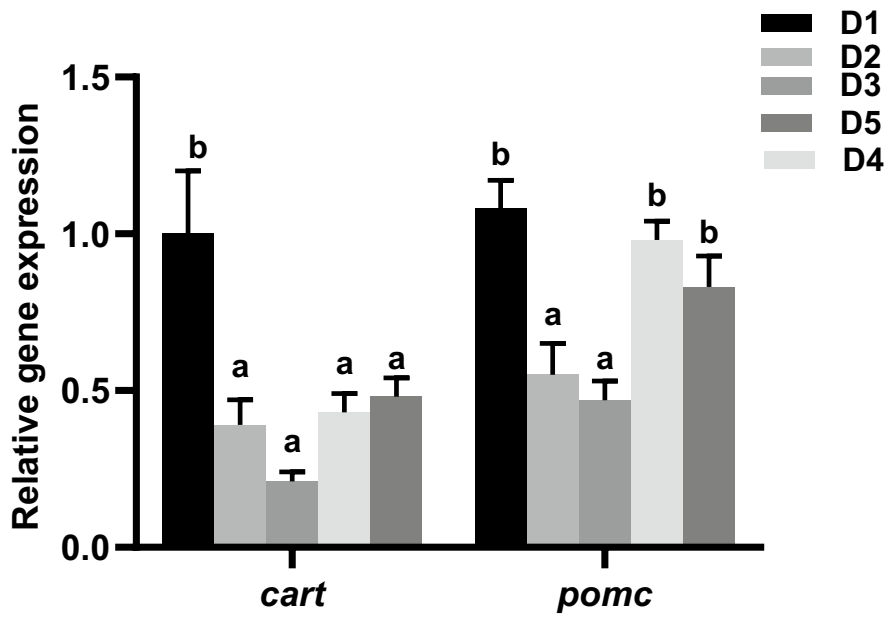

Fig. 5 The mRNA expression levels of genes involved in appetite-inhibiting genes in the hypothalamus (*cart*, *cocaine-and amphetamine-regulated transcript*; *pomc*, *proopiomelanocortin*). Values are presented as the means ± SEM ($n = 6$). Different lowercase letters above the bars indicate significant difference ($P < 0.05$)

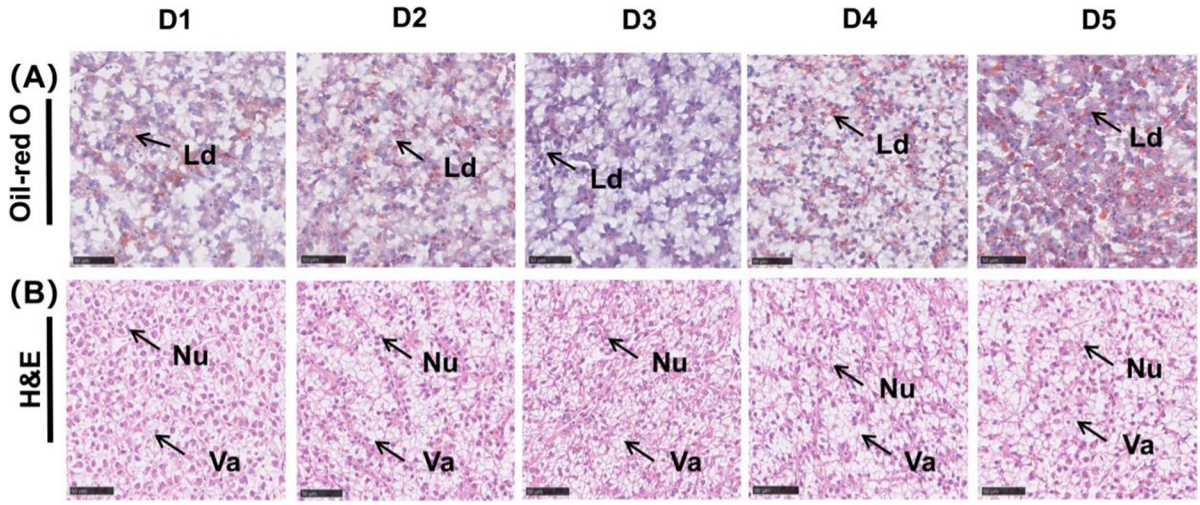

Fig. 6 Histology analyses of liver section and determination of hepatocyte inflammation. **A** Hepatic tissue Sect. (40 × magnification) of Oil Red O staining of Chinese perch fed with dietary different CHO:L level. **B** Hepatic tissue Sect. (40 × magnification) of hematoxylin and eosin staining (H&E) 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

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). 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 the 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, 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 (Brauge et al 1995; Hemre et al. 2002; Li et al 2019a, b, c, d).

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 (Li, et al 2019a, b, c, d) and golden pompano (*Trachinotus ovatus*) (Dong et 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*) (Wang et 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 (Ren 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 (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 (Li et al 2012; Lu 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 (SitjÀ-Bobadilla and PÉRez-SÁNchez 1999) and blunt snout bream(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 (Shearer et al 2012; Lu 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 *accα* 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 the 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; Egea et 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 the 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 the 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 (Li et al 2019a, b, c, d; Conde-Sieira and Soengas 2016). In previous studies, fish's appetite was influenced by the nutritional content of the feed and the proportion of different ingredients added. By Basto-Silva (Basto-Silva et al 2021), studies have shown that different dietary protein to energy ratio affects the feeding intake and appetite regulation of gilthead seabream (*Sparus aurata*). 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 (Blouet and Schwartz 2009). These circuits mainly include *npy/agrp*, *pomc/cart* neurons and central and peripheral endocrine factors that respond to circulating glucose, fatty acid, or amino acid levels, respectively (Efeyan et al 2015; Conde-Sieira and Soengas 2016; Mobbs et al 2005). In previous studies based on Chinese perch in our laboratory, central nervous system *npy/agrp* was generally used as an appetite promoting factor, while *pomc/cart* was used as an appetite suppressant factor (Liu et al 2020). 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 *npy/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 *npy* and *agrp* 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 (Castro et al 2015), rainbow trout (Otero-Rodiño et al 2016), and Siberian sturgeon (*Acipenser baerii*) (Gong et al 2015) reduced food intake. High lipid diets also decrease food intake of fish (Dai 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.

The liver is an indicator organ to measure the nutritional and physiological status of fish, and nutritional imbalance can cause changes in the histological status of the cells(Wang et al 2014). The determination of nutrient requirements cannot be based only on the growth indicators of experimental animals, but also on their health and pathological reactions (Kumar et al 2005). Previous studies have shown that excessive dietary fat and carbohydrate content can cause metabolic burden of fish, resulting in a series of pathological reactions such as hepatic hypertrophy, hepatic steatosis, apoptosis, hepatocyte irregular arrangement, and fatty liver (Ishak et al 2016; Lu et al 2013). In our study, it was found that when fed with different carbohydrate-lipid ratio diets, liver H&E staining sections indicated that the number of hepatic

cell vacuoles in D3 group was lower than that in other groups and so was the number of lipid droplets in D3 group. This indicated that the liver health status was better when the carbohydrate-to-lipid ratio was 1.71.

Conclusion

In conclusion, through broken line regression analysis, the optimal CHO:L ratio in the diet of Chinese perch is recommended to be 1.60. The CHO:L ratio 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 CHO:L ratio 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.

Author contribution 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. J. L., S-L T., K. L., and Q-W Z.: revised the manuscript.

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Data availability All data are available from the corresponding author by request.

Code availability Not applicable.

Declarations

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

Consent to participate Not applicable.

Consent for publication All authors review and approve the manuscript for publication.

Conflict of interest The authors declare no competing interests.

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