Animal Nutrition 19 (2024) 429–441

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary carbohydrate to lipid ratio affects growth, reproductive performance and health of female yellow catfish (*Pelteobagrus fulvidragrus*): A lipidomics analysis

Shuzhan Fei [a, b, c], Zheng Chen [a, b], Haokun Liu [a], Junyan Jin [a], Yunxia Yang [a], Dong Han [a, b, d, *], Xiaoming Zhu [a, b, *], Shuoqi Xie [a, b, e]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[b] College of Advanced Agricultural Sciences, University of Chinese Academy of Sciences, Beijing 100049, China
[c] Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou, Guangdong 510380, China
[d] Hubei Hongshan Laboratory, Wuhan 430070, China
[e] The Innovative Academy of Seed Design, Chinese Academy of Sciences, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 30 March 2024
Received in revised form
26 June 2024
Accepted 5 July 2024
Available online 28 August 2024

Keywords:
Yellow catfish
Carbohydrate
Lipid
Reproductive performance
Lipidomics

ABSTRACT

This study aimed to examine the impact of dietary carbohydrate to lipid (CHO/L) ratio on the growth, reproductive, and offspring performance of broodstock yellow catfish, and to elucidate the metabolic differences between mothers and offspring using lipidomics. Five isonitrogenous and isoenergetic diets with varying CHO/L ratios (0.65, 1.44, 2.11, 3.13, and 5.36) were fed to five groups of female broodfish with three replicates per group and 35 female broodfish per replocate in a pond-cage culture system. After an eight-week feeding trial, the dietary CHO/L ratio had a significant impact on the growth and reproductive performance of female yellow catfish. The weight gain ratio (WGR) and specific growth rate (SGR) in the CHO/L0.65 and CHO/L2.11 groups were significantly higher than those in the CHO/L5.36 group ($P < 0.05$). The fertilization and hatching rates were the highest when the dietary CHO/L ratio was 0.65 and 2.11, respectively. When the dietary CHO/L ratio was 3.13 and 5.36, the plasma contents of testosterone (T) was significantly lower than those of other groups ($P = 0.013$), and the plasma vitellogenin (VTG) content was the lowest when the CHO/L ratio was 5.36. The plasma contents of estradiol (E2) significantly decreased with increasing dietary CHO/L ratio ($P_L = 0.012$). Lipidomic analysis revealed that the ovary primarily consisted of five subclasses in terms of lipid composition, namely triglyceride, fatty acyl, sterol, glycerophospholipid, and sphingolipid; however, sphingolipids were not detected in the larvae. The relative expression levels of the ovarian lipid metabolism-related genes sterol regulatory element binding protein 1 (*srebp1*), acetyl-CoA carboxylase (*acc*), delta (12)-oleate desaturase (*fad2*), and elongation of very long chain fatty acids protein 5 (*elvol5*) significantly increased with increasing dietary CHO/L ratio ($P < 0.05$). The relative expression levels of lipid metabolism-related genes *srebp 1*, peroxisome proliferator activated receptor α (*ppar*α), carnitine palmitoyl transferase 1 isoform (*cpt*), adipose triglyceride lipase (*atgl*), *fad2*, and *elvol5* in offspring larvae were initially increased and then decreased with increasing dietary CHO/L ratios until reaching a maximum at a ratio of 2.11 ($P < 0.05$). In conclusion, based on the broken-line regression of the dietary CHO/L ratio and egg diameter, the optimal dietary CHO/L ratio was 1.91 for broodfish yellow catfish. A high CHO/L ratio diet results in increased lipogenesis

* Corresponding authors.
E-mail addresses: hand21cn@ihb.ac.cn (D. Han), xmzhu@ihb.ac.cn (X. Zhu).
Peer review under the responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2024.07.003

and hepatic lipid accumulation in maternal organisms, leading to impaired reproductive performance and reduced offspring quality.

1. Introduction

The development of cost-effective and nutrient-adequate formulated feed is essential for ensuring the viability of sustainable fish farming and propagation. Incorporating both carbohydrates and lipids into fish diets maximizes the efficient use of dietary protein while providing essential non-protein energy sources required for growth and overall health in aquatic organisms. Carbohydrates, as the most cost-effective energy source in aquatic feed, can reduce the protein requirement for fish at appropriate levels (Zhou et al., 2016), thereby minimizing the consumption of protein as an energy source—a phenomenon known as protein sparing (NRC, 2011). Carbohydrates also participate in the synthesis of non-essential amino acids and lipids (Zhou et al., 2013). However, fish exhibit limited capacity for carbohydrate utilization, with carnivorous species displaying inadequate ability to digest and metabolize carbohydrates. Indeed, excessive carbohydrate intake in carnivorous fish diets can negatively impact growth performance and impair various physiological functions, such as elevated blood glucose levels, liver glycogen accumulation, hepatic dysfunction, and immunosuppression (Lin et al., 2018; Ren et al., 2011).

Compared to carbohydrates, lipids are highly utilized by most fish species. Lipids serve as an energy source and provide essential fatty acids, intact phospholipids, and cholesterol necessary for normal growth, development, and overall health (Luo et al., 2005). However, excessive dietary lipid intake may increase susceptibility to autoxidation and tissue lipid peroxidation, adversely affecting fish health (Lu et al., 2014). Additionally, excessive lipid ingestion can disrupt the digestible energy/crude protein ratio, leading to abnormal fat deposition in fish (Chou et al., 2001). Our laboratory has investigated the lipid requirements of broodstock yellow catfish, achieving optimal growth and reproductive performance with dietary lipid levels ranging between 10.1% and 11.7% (Fei et al., 2023). Therefore, it is essential to provide an appropriate proportion of non-protein energy sources (carbohydrates and lipids) in farmed fish feed, as both play crucial regulatory roles in physiological processes such as growth and reproduction. Research in mammals has shown that high maternal carbohydrate or fat intake can increase the risk of offspring developing metabolic syndrome, disrupting lipid metabolism, causing insulin resistance, and obesity symptoms in the subsequent generation, suggesting intergenerational genetic effects (Huang et al., 2017; Quiclet et al., 2017). However, there is a scarcity of studies investigating the optimal carbohydrate to lipid (CHO/L) ratio requirements of parental fish. However, few studies have investigated the optimal CHO/L ratio requirements for parent fish.

The yellow catfish (*Pelteobagrus fulvidraco*) is a highly valued species in East and South Asia because of its excellent meat quality and flavor. The rapid expansion of yellow catfish in China's aquaculture industry has been greatly stimulated, leading to an annual production of over 600,000 metric tons by 2023 (FBMA, 2023). The development of the ovary requires the accumulation of a large amount of nutrients to ensure the normal development of embryos and early larvae; therefore, ensuring adequate parental nutrition is the key to obtaining high-quality offspring (Izquierdo et al., 2001). Lipidomics is widely used in the study of many metabolic disorders (Wenk, 2005), and provides an accurate depiction of the comprehensive lipid profile within a cell or tissue, including its structures, functionalities, interactions, and dynamics (Liu et al., 2019). However, few studies have used lipidomics to investigate the effects of maternal nutrition on lipid metabolism in mothers and their offspring during the reproductive period. Therefore, the purpose of this study was to investigate the optimal dietary CHO/L ratio for female yellow catfish, evaluate the effects of dietary carbohydrates and lipids on reproductive performance and egg quality, and elucidate the potential mechanisms underlying different CHO/L ratio diets on maternal and offspring larval performance through lipidomic analysis.

2. Materials and methods

2.1. Animal ethics statement

All fish experiments were approved by the Institute of Hydrobiology, Chinese Academy of Sciences (Approval ID: IHB 2013724).

2.2. Experimental diets

Five isonitrogenous and isoenergetic diets were formulated to contain five graded CHO/L ratios ranging from 0.65 to 5.36 by adjusting the lipid and carbohydrate (nitrogen-free extract) contents. The gross energy content of the diets was calculated based on 23.7, 39.5 and 17.2 kJ/g for protein-, lipid-, and nitrogen-free extract, respectively. White fishmeal, corn gluten meal, and casein were used as the protein sources. Fish oil was used as the lipid source. Corn starch was used as the carbohydrate source. The feed formulations and their proximate compositions are listed in Table 1. All ingredients were accurately weighed and thoroughly mixed before being ground into a fine powder using a mill with a 100-μm mesh. The dough containing water was passed through a laboratory-scale pelleter to 4.0-mm pellets. The pellets were then dried at 80 °C for 40 min in an oven. Finally, the pellets were sieved and evenly sprayed with fish oil that had been weighed beforehand in a blender. All feeds were stored at 4 °C until further use.

2.3. Experimental fish and management

The same batch of two winter-aged female yellow catfish was used, and all the fish were temporarily raised in a pond cage culture system of the Fisheries Research Center of Guangdong Haid Group Co. Ltd., China and fed with commercial feed (crude protein: 45% and crude lipid: 10.0%) twice a day for 2 weeks to ensure that the experimental fish could adapt to the culture environment. Before the formal experiment, a total of 525 fish (initial average weight: 64.43 ± 0.34 g) were starved for 24 h and weighed, then they were randomly distributed to 15 net cages (1.0 m × 1.0 m × 2.0 m) in the same pond. Each cage was stocked with 35 broodfish and each experimental feed was randomly assigned to triplicate cages. The fish were hand-fed to satiation twice daily at 07:30 and 17:30, for 8 weeks. During the feeding trial, the level of dissolved oxygen in the water was 5.5 to 8.9 mg/L, the pH ranged from 6.9 to 7.9, and the

Table 1
Composition and nutrient levels of diets (dry matter basis, %).

Item	Dietary CHO/L ratios				
	0.65	1.44	2.11	3.13	5.36
Ingredients					
White fishmeal	33.00	33.00	33.00	33.00	33.00
Corn gluten meal	7.00	7.00	7.00	7.00	7.00
Casein	17.00	17.00	17.00	17.00	17.00
Corn starch	12.00	16.50	21.00	25.50	30.00
Fish oil	11.60	9.20	6.80	4.40	2.00
Vitamin premix[1]	0.39	0.39	0.39	0.39	0.39
Mineral premix[2]	5.00	5.00	5.00	5.00	5.00
CMC	2.00	2.00	2.00	2.00	2.00
$Ca(H_2PO_4)_2$	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.11	0.11	0.11	0.11	0.11
Ethoxyquin	0.10	0.10	0.10	0.10	0.10
Cellulose	10.80	8.70	6.60	4.50	2.40
Total	100.00	100.00	100.00	100.00	100.00
Nutrient levels					
Crude protein	42.01	42.51	42.11	41.95	41.86
Crude lipid	15.76	12.05	10.31	8.50	6.12
Crude fiber	18.37	16.06	13.63	10.70	7.71
Moisture	5.84	4.54	4.99	4.32	4.43
Ash	7.82	7.51	7.21	7.89	7.14
Nitrogen free extract[3]	10.20	17.33	21.75	26.64	32.75
CHO/L ratio[4]	0.65	1.44	2.11	3.13	5.36
Gross energy, kJ/g	18.02	17.87	18.11	17.94	17.98

CMC = sodium carboxymethyl cellulose; CHO/L = carbohydrate/lipid.
[1] Vitamin premix (mg/kg diet): vitamin B_1 20, vitamin B_2 20, vitamin B_6 20, vitamin B_{12} 0.02, folic acid 5, calcium pantothenate 50, inositol 100, niacin 100, biotin 0.1, cellulose 5561.88, ascorbic acid 4000, vitamin A 11, vitamin D 2, vitamin E 100, vitamin K 10.
[2] Mineral premixes (mg/kg diet): NaCl 500.0, $MgSO_4 \cdot 7H_2O$ 8155.6, $NaH_2PO_4 \cdot 2H_2O$ 12,500.0, KH_2PO_4 16,000.0, $Ca(H_2PO_4) \cdot 2H_2O$ 7650.6, $FeSO_4 \cdot 7H_2O$ 2286.2, $C_6H_{10}CaO_6 \cdot 5H_2O$ 1750.0, $ZnSO_4 \cdot 7H_2O$ 178.0, $MnSO_4 \cdot H_2O$ 61.4, $CuSO_4 \cdot 5H_2O$ 15.5, $CoSO_4 \cdot 7H_2O$ 0.91, KI 1.5, Na_2SeO_3 0.60, corn starch 899.7.
[3] Nitrogen free extract = 100 − crude protein − crude lipid − crude cellulose − Ash.
[4] CHO/L = nitrogen free extract/crude lipid on the weight basis of dry matter.

ammonia nitrogen content was less than 0.5 mg/L. The water temperature rose steadily from 24 to 32 °C between March until June.

2.4. Artificial reproduction process

Nine males used for artificial insemination were sourced from a homogenous cohort of parents reared in an identical pond environment. After the feeding trial, nine females from each treatment group were randomly selected for breeding. The "semi-dry" artificial propagation technology was implemented, and the operational procedures and assessment of reproductive performance were the same as our previous research method (Fei et al., 2023). In brief, the female was injected with 20 ng of domperidone (DOM), 16 μg of luteinizing hormone-releasing hormone-A2 (LHRH-A2), and 1500 IU of human chorionic gonadotropin (HCG) per kilogram body weight. The dose was administered in two rounds, with one-fourth administered initially and the remaining three-quarters injected after a 10-h interval. Male fish were injected with the same hormones, but at half the dosage used for females. Upon ovulation, eight male fish were anesthetized with MS-222 (80 mg/L; Sigma, USA) and dissected to extract their testes. Semen was obtained using a mortar and preserved in a sperm preservative solution at 10 times dilution. Subsequently, eggs were collected, approximately 2 mL of semen was added to the eggs, followed by approximately 30 mL of saline, and the artificial insemination process was completed by stirring with a soft brush for 20 s. The number of eggs (approximately 0.5 g) was counted to determine the total number of eggs produced by the broodstock. The fertilization rate was measured as follows: approximately 200 eggs from each fish were randomly selected and placed into 10-cm diameter Petri dishes. Each batch of eggs was incubated in 4-L plastic containers maintained at 28 °C. Upon hatching, the number of larvae was counted to calculate the hatching rate. The diameter of at least 30 eggs was measured for each fish species. The 3 days post-hatching (3 DPH) larvae were collected and stored at −80 °C for lipid metabolite extraction (three replicates in each group).

Reproductive performance was calculated according to the following formulae:

Fertilization rate (%) = 100 × total fertilized eggs/total number of eggs;

Hatching rate (%) = 100 × total number of larvae/total number of fertilized eggs;

Malformation rate (%) = 100 × total malformed larvae/total number of larvae;

Absolute fecundity = total egg produced per female;

Relative fecundity = total egg production per female/mean weight of female.

2.5. Sample collection

Following the feeding trial, all fish were subjected to a 24 h fasting period. The fish in each cage were anesthetized with MS-222 (80 mg/L; Sigma, USA), counted, and weighed. Two fish were collected from each cage to determine the chemical composition of the whole body. Three fish were randomly chosen from each cage, and the ovary, liver, and visceral masses were dissected and weighed to calculate the gonadsomatic index (GSI), hepatosomatic index (HSI), and viscerosomatic index (VSI). In addition, two fish from each cage were individually sampled to withdraw blood serum and then dissected to collect the liver and ovary tissues for real-time quantitative PCR (RT-qPCR) and lipidomics analysis. A piece of liver tissue was preserved in 4% paraformaldehyde solution for liver pathological sectioning.

Growth performance was calculated according to the following formulae:

Weight gain ratio (WGR, %) = 100 × (final body weight - initial body weight)/initial body weight;

Feed efficiency (FE, %) = 100 × weight gain (wet weight)/feed consumed (dry weight);

Specific growth rate (SGR, %/d) = 100 × [ln (initial body weight) - ln (final body weight)]/days;

Feed intake (FI, %/d) = 100 × feed consumption/[days × (final body weight + initial body weight)/2];

HSI (%) = 100 × liver weight/whole body weight;

VSI (%) = 100 × viscera weight/whole body weight;

GSI (%) = 100 × ovary weight/body weight.

2.6. Biochemical analysis

The chemical compositions of the feed and fish (moisture, ash, crude protein, crude fiber, and crude lipids) were analyzed following official methods (AOAC, 2006). Dry matter content was determined by drying samples at 105 °C for 24 h until a constant weight was achieved, and then calculated as the percentage of water loss (AOAC, 2006; method 930.15). The crude protein was calculated by multiplying nitrogen by the factor 6.25. The nitrogen was measured according to the method 984.13 of AOAC (2006). The crude lipid and ash contents were measured according to method 920.39 and 942.05 (AOAC, 2006), respectively. Crude fiber was determined by an automatic fiber analyzer (Ankom200, ANKOM, USA) according to the method 978.10 of AOAC (2006). An automatic oxygen bomb calorimeter (Gentry Instrument Inc., Aiken, USA) was used to measure the gross energy (GE) of feed. The levels of plasma testosterone (T), 17-β estradiol (E2) and vitellogenin (VTG) were quantified using Elisa Kits according to the manufacturer's recommendations (H090-1-2, H102-1-2 and H362-1, Nanjing Jiancheng Bioengineering Institute, China).

2.7. Histological analysis

The liver tissue was fixed in 4% paraformaldehyde for 48 h, then washed twice with phosphate buffer saline (PBS). Subsequently, the tissue was immersed in a 30% sucrose solution (prepared with PBS) at 4 °C for 12 h and then embedded in optimal cutting temperature compound. Continuous sections of 8 μm thickness were obtained using a cryotome. These sections were fixed with a fixative solution and stained with neutral Oil Red O to visualize the accumulation of lipid droplets in the liver. Microscopic examination was performed to assess the staining effect and the slides were sealed with gelatin. For routine paraffin embedding, liver samples were fixed and processed. Wax sections of 6 μm thickness were stained using hematoxylin-and-eosin (H&E). Microscopic and image-acquisition analyses were performed using a microscope (Axio Imager A2, Zeiss, Germany).

2.8. Lipid metabolite extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of ovary and 3 DPH larvae

Ovary and 3 DPH larvae were thawed at 4 °C on ice. Multi-point samples were weighed to 20 mg, then homogenized (30 Hz) for 20 s using a steel ball and the centrifuge (3000 × *g*, 4 °C) for 30 s. Subsequently, 1 mL of the extraction solvent (MTBE: MeOH = 3:1, v/v) containing an internal standard mixture was added. After mixing for 15 min, 200 μL of water was added. The mixture was vortexed for 1 min and centrifuged at 13,400 × *g* for 10 min. Following this, 200 μL of the upper organic layer was retrieved and evaporated using a vacuum concentrator. The dried extract was reconstituted with 200 μL of mobile phase B prior to LC-MS/MS analysis. Details of the lipidomics based on LC-MS/MS can be found in the supporting information.

2.9. RNA extraction, reverse transcription and mRNA level analysis

Total RNA was isolated from the liver and 3 DHP larvae ($n = 6$) using TRIzol reagent (9109, TaKaRa, Japan). The M-MLV First-Strand Synthesis Kit (28025-013, Invitrogen, China) was used for the reverse transcription of cDNA. The primer sequences for fatty acid synthase (*fas*), sterol regulatory element binding protein 1 (*srebp1*), acetyl-CoA carboxylase (*acc*), lipoprotein lipase (*lpl*), peroxisome proliferator activated receptor α (*pparα*), carnitine palmitoyl transferase 1 isoform (*cpt1*), hormone-sensitive lipase (*hsl*), adipose triglyceride lipase (*atgl*), elongation of very long chain fatty acids protein 5 (*elvol5*), delta (12)-oleate desaturase (*fad2*) and the housekeeping gene are presented in Table 2. The RT-qPCR was conducted using a Light Cycler 480 II System (Roche, Switzerland). The RT-qPCR program was as follows: 5 min preincubation at 95 °C, 40 cycles with 10 s at 95 °C, 20 s at melting temperature, and 10 s at 75 °C. The mRNA transcriptional levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2001).

2.10. Data processing

Statistical analysis of all data obtained in this experiment was conducted using SPSS 23.0 (IBM, USA) software, and the results are presented as the mean ± standard error (mean ± SE). Normality and homogeneity of variance were confirmed before conducting one-way analysis of variance (ANOVA). Duncan's multiple range test was used to identify significant differences among the groups, with a significance threshold of $P < 0.05$. Lipidomic data were subjected to multivariate statistical analysis using SIMCA-P14.1 (Umetrics, Umea, Sweden). This included unsupervised principal component analysis (PCA) and supervised orthogonal partial least squares discriminant analysis (OPLS-DA), which were employed for classification modeling and the simultaneous extraction of variable importance for the projection (VIP) values > 1. For OPLS-DA analysis and heat map generation, R project software (www.r-project.org/) was used.

3. Results

3.1. Growth, morphological indexes and proximate composition of whole body

As shown in Table 3, WGR and SGR in the CHO/L0.65 and CHO/L2.11 groups were significantly higher than those in the CHO/L5.36 group ($P < 0.05$). Broodstock fed diets with CHO/L ratios of 1.44 and 2.11 exhibited a higher GSI than those fed the other diets ($P = 0.039$). However, FE, HSI, and VSI were not significantly influenced by dietary CHO/L ratio (Table 3). No significant differences were observed in the crude protein and crude lipid content of the whole body among the dietary groups. Fish fed a diet with a CHO/L ratio of 2.11 exhibited the highest lipid content in the ovary, whereas the muscle had the lowest lipid content (Table 4).

3.2. Reproductive performance

As shown in Table 5, fertilization and hatching rates were significantly higher in fish fed diets with CHO/L ratios of 0.65 and 2.11 compared with all other groups ($P < 0.05$). Fish fed a diet with a CHO/L ratio of 5.36 exhibited significantly higher malformation rates than those observed in other groups ($P = 0.015$). Both the absolute and relative fecundity of the broodstock showed a consistent trend, decreasing significantly with increasing dietary CHO/L ratio ($P_L = 0.025$ and $P_L = 0.029$, respectively). Regression analysis between egg diameter and the dietary CHO/L ratio showed a suitable dietary CHO/L ratio of 1.91 (Fig. 1). The body length of the 3 DPH larvae declined significantly with increasing dietary CHO/L ratios ($P_L < 0.001$ and $P_Q < 0.001$, respectively).

3.3. Plasma steroid hormones and vitellogenin

As shown in Table 6, linear regression analysis showed that plasma T and E2 contents decreased significantly with the increase of CHO/L ratio ($P_L = 0.001$ and $P_L = 0.012$, respectively). The content

Table 2
Primers used for real-time quantitative PCR analysis.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to3′)	GenBank no.	Annealing temperature, °C	Amplicon size, bp
fas	AACTAAAGGCTGCTGGTTGCTA	CACCTTCCCGTCACAAACCTC	MH253823.1	60	141
srebp1	CTGGGTCATCGCTTCTTTGTG	TCCTTCGTTGGAGCTTTTGTCT	JX992742.1	60	189
acc	GGGGTTTTCACGCTGCTTC	GGTTCTGATTGGGTCGTCCTG	JX992746.1	60	163
lpl	CTCCATCCACCTCTTCATC	CTTTGTACGGCATCATCTC	JX992743.1	56	254
pparα	ATCCAGGAATCCAGCACAA	GCACATACACGGCACTCAA	JX992740.1	56	149
cpt1	GAGCGATTGGTGGGAGGAG	CTGGGATGCGACTGGTGTT	JQ074178.1	60	257
hsl	TCACGGTTTCTCACGCTTAT	GATGTAGGAGTGCGGTCAA	KJ588764.1	58	215
atgl	TTGCGGAAATGTGATTGAGGT	CACGGAAGGCAGGAGGGA	KF614123.1	60	132
elvol5	ACGGAGGCTAGAAATGGA	CAGGAGGTAAATGATGGTGA	XM027165677.1	60	145
fad2	CTTGCGTAAGAGGTTGGA	TAAAGACTGACAGGTGGC	XM027173335.1	55	221
β-Actin	GAAGGTTATGCTCTGCCCCAT	GTGAAGCTGTAGCCTCTCTCG	XM027148463	60	138

fas = fatty acid synthase; *srebp1* = sterol regulatory element binding protein 1; *acc* = acetyl-CoA carboxylase; *lpl* = lipoprotein lipase; *pparα* = peroxisome proliferator activated receptor α; *cpt1* = carnitine palmitoyl transferase 1 isoform; *hsl* = hormone-sensitive lipase; *atgl* = adipose triglyceride lipase; *elvol5* = elongation of very long chain fatty acids protein 5; *fad2* = delta (12)-oleate desaturase.

Table 3
Effects of different dietary CHO/L ratios on growth and morphometric indexes of female yellow catfish broodstock.

Item	Dietary CHO/L ratios					*P*-value		
	0.65	1.44	2.11	3.13	5.36	ANOVA	Linear	Quadratic
IBW, g	64.50 ± 0.064	64.50 ± 0.086	64.56 ± 0.052	64.63 ± 0.080	64.49 ± 0.083	–	–	–
FBW, g	87.24 ± 0.716^{b}	85.17 ± 2.461^{ab}	86.02 ± 0.263^{b}	84.21 ± 1.472^{ab}	79.18 ± 2.778^{a}	0.047	0.011	0.023
WGR, %	35.25 ± 1.176^{b}	32.04 ± 4.063^{ab}	33.23 ± 0.312^{b}	30.31 ± 2.355^{ab}	22.76 ± 4.212^{a}	0.038	0.010	0.022
FE, %	60.02 ± 7.365	53.48 ± 6.218	53.48 ± 6.764	59.43 ± 5.201	47.70 ± 10.012	0.734	0.400	0.695
SGR, %/d	0.70 ± 0.020^{b}	0.64 ± 0.072^{ab}	0.67 ± 0.005^{b}	0.61 ± 0.042^{ab}	0.47 ± 0.081^{a}	0.048	0.011	0.023
FI, %/d	0.85 ± 0.048	0.85 ± 0.034	0.83 ± 0.022	0.79 ± 0.021	0.73 ± 0.034	0.070	0.004	0.062
HSI, %	1.99 ± 0.109	1.90 ± 0.102	1.87 ± 0.052	2.16 ± 0.088	2.09 ± 0.107	0.203	0.128	0.209
VSI, %	29.30 ± 0.845	29.54 ± 0.654	29.28 ± 0.893	28.83 ± 0.806	27.51 ± 0.656	0.342	0.072	0.103
GSI, %	13.24 ± 0.760^{ab}	14.25 ± 0.799^{b}	14.04 ± 1.011^{b}	12.74 ± 1.085^{ab}	11.20 ± 0.433^{a}	0.039	0.042	0.022

CHO/L = carbohydrate/lipid; IBW = initial body weight; FBW = final body weight; WGR = weight gain ratio; FE = feed efficiency; SGR = specific growth rate; FI = feed intake; HSI = hepatosomatic index; VSI = viscerosomatic index; GSI = gonadsomatic index.
^{a,b} Values in the same row with different letter superscripts are significantly different ($P < 0.05$) .Values are presented as mean ± SE, $n = 3$ (for 3 replicate groups, 6 fish per replicate).

Table 4
Effects of different dietary CHO/L ratios on proximate composition (%) of whole body and tissues of female yellow catfish broodstock.

Item	Dietary CHO/L ratios					*P*-value		
	0.65	1.44	2.11	3.13	5.36	ANOVA	Linear	Quadratic
Crude protein	15.02 ± 0.841	14.38 ± 0.880	15.99 ± 1.051	17.60 ± 0.573	15.87 ± 0.658	0.098	0.082	0.226
Crude lipid	16.51 ± 1.176	15.59 ± 2.055	16.36 ± 1.293	17.33 ± 0.655	14.91 ± 1.404	0.772	0.730	0.864
Ovary lipid	11.88 ± 0.388^{a}	12.59 ± 0.342^{b}	14.23 ± 1.092^{c}	12.33 ± 0.134^{ab}	12.67 ± 0.204^{b}	<0.001	0.456	0.062
Muscle lipid	8.82 ± 0.829	9.56 ± 1.351	8.03 ± 0.352	9.25 ± 0.613	7.89 ± 0.494	0.209	0.408	0.621

CHO/L = carbohydrate/lipid.
$^{a-c}$ Values in the same row with different letter superscripts are significantly different ($P < 0.05$) . Values are presented as mean ± SE ($n = 6$).

Table 5
Effects of different dietary CHO/L ratios on reproductive performance of female yellow catfish.

Item	Dietary CHO/L ratios					*P*-value		
	0.65	1.44	2.11	3.13	5.36	ANOVA	Linear	Quadratic
Fertilization rate, %	65.13 ± 12.502^{b}	43.02 ± 4.698^{ab}	59.56 ± 7.833^{b}	35.72 ± 2.543^{a}	55.86 ± 7.314^{ab}	0.002	0.105	0.105
Hatching rate, %	76.31 ± 3.734^{c}	59.42 ± 5.020^{b}	74.61 ± 4.478^{c}	56.57 ± 5.214^{b}	37.19 ± 3.842^{a}	0.001	<0.001	0.001
Malformation rate, %	9.12 ± 0.615^{ab}	8.17 ± 0.683^{a}	10.43 ± 1.375^{ab}	8.83 ± 1.444^{ab}	11.79 ± 0.861^{b}	0.015	0.090	1.171
Absolute fecundity	10,324.61 ± 838.821^{b}	9008.92 ± 536.344^{ab}	9083.92 ± 626.014^{ab}	9305.82 ± 760.205^{ab}	6570.24 ± 433.301^{a}	0.049	0.025	0.064
Relative fecundity	112.01 ± 12.061^{b}	100.34 ± 5.890^{ab}	93.53 ± 9.552^{ab}	97.9 ± 12.563^{ab}	80.67 ± 4.665^{a}	0.044	0.029	0.095
Egg diameter, mm	1.23 ± 0.011^{b}	1.23 ± 0.014^{b}	1.25 ± 0.028^{b}	1.19 ± 0.052^{ab}	1.14 ± 0.019^{a}	0.038	0.013	0.012
Larvae body length 3 DPH, mm	7.65 ± 0.005^{b}	7.33 ± 0.008^{ab}	7.31 ± 0.006^{ab}	7.19 ± 0.005^{ab}	6.98 ± 0.005^{a}	0.031	<0.001	<0.001

CHO/L = carbohydrate/lipid; DPH = day post-hatching.
$^{a-c}$ Values in the same row with different letter superscripts are significantly different ($P < 0.05$) . Values are presented as mean ± SE ($n = 9$).

of plasma VTG initially increased and then decreased with increasing dietary CHO/L ratio ($P_Q < 0.001$), the VTG content in the CHO/L5.36 group being significantly lower than that in other groups ($P = 0.014$).

3.4. Histological changes

The liver histology and histochemistry observations of the yellow catfish in this study are shown in Fig. 2. The results demonstrated

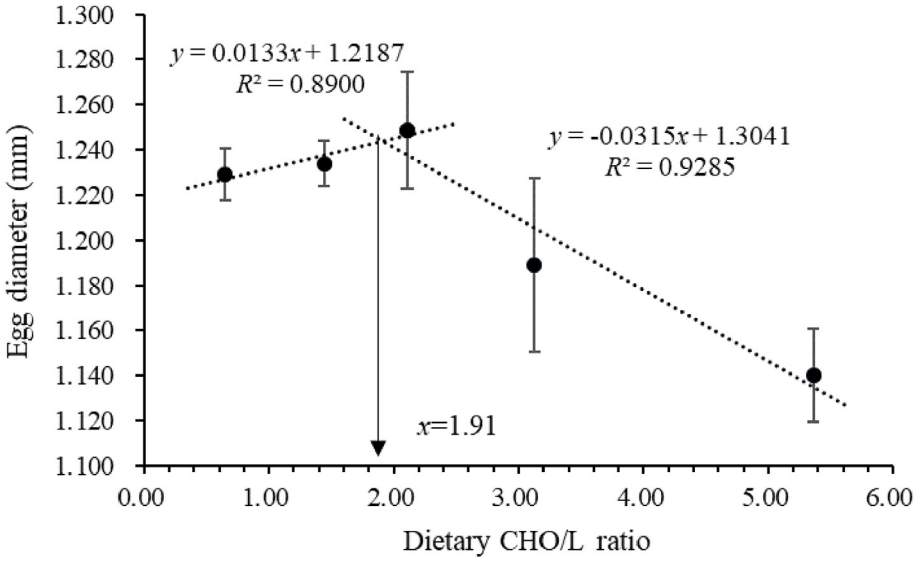

Fig. 1. Broken-line regression analysis of egg diameter against varying ratios of dietary CHO/L. Data are shown as mean ± SE (n = 30). CHO/L = carbohydrate/lipid.

Table 6
Effects of dietary CHO/L ratios on plasma sex hormone levels of female yellow catfish.

Item	Dietary CHO/L ratios					*P*-value		
	0.65	1.44	2.11	3.13	5.36	ANOVA	Linear	Quadratic
Testosterone, mmol/L	50.63 ± 8.813^{b}	50.0 ± 4.031^{b}	48.00 ± 6.084^{b}	22.32 ± 4.319^{a}	16.55 ± 4.114^{a}	0.013	0.001	<0.001
Estradiol, ng/L	108.72 ± 16.944^{bc}	114.07 ± 18.670^{c}	88.71 ± 19.012^{abc}	51.38 ± 10.213^{a}	59.39 ± 12.690^{ab}	0.025	0.012	<0.001
Vitellogenin, μg/L	30.65 ± 6.571^{ab}	38.83 ± 8.732^{b}	32.52 ± 5.633^{b}	36.42 ± 6.124^{b}	12.34 ± 2.290^{a}	0.014	0.026	<0.001

CHO/L = carbohydrate/lipid.
$^{a\text{-}c}$ Values in the same row with different letter superscripts are significantly different ($P < 0.05$) . Values are presented as mean ± SE (n = 6).

that an increase in the CHO/L ratio led to pronounced hepatic vacuolization, accompanied by the augmentation of both the area and deposition of liver lipid droplets. This observation was consistent with the quantification of the lipid droplet area in the sections (Table 7) and was further validated by the liver lipid content (Table 7). These results indicated that a low-fat/high-carbohydrate diet is more likely to induce hepatic lipid accumulation than a high-fat/low-carbohydrate diet.

3.5. Overall change of lipid metabolites in ovary and offspring larvae

As shown in Fig. 3. OPLS-DA was used to analyze the contribution of lipids to the separation of ovaries and offspring larvae in response to different dietary CHO/L ratios of 0.65, 2.11, and 5.36. R^2Y represents the percentage of variation explained by the model and Q^2 represents the predictive ability of the model. The OPLS-DA

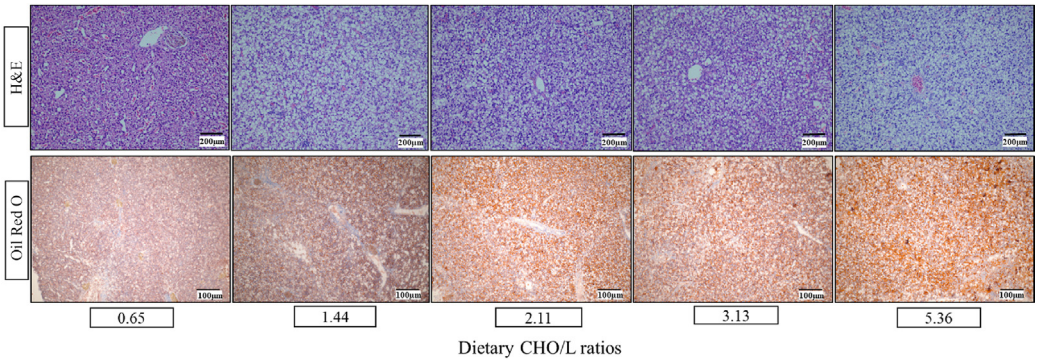

Fig. 2. Liver hematoxylin-and-eosin (H&E) and Oil Red O staining sections after feeding different CHO/L diets to female yellow catfish. The relative areas of lipid droplets in Oil Red O staining were quantified and expressed as a percentage of the total area, with the red-colored lipid droplets uniformly converted. CHO/L = carbohydrate/lipid.

Table 7
Effects of dietary CHO/L ratios on liver lipid content and relative areas (%) for lipid droplets in liver Oil Red O staining of female yellow catfish.

Item	Dietary CHO/L ratios					*P*-value		
	0.65	1.44	2.11	3.13	5.36	ANOVA	Linear	Quadratic
Liver lipid	12.64 ± 1.016[a]	16.63 ± 1.171[ab]	17.84 ± 0.712[b]	17.39 ± 0.485[b]	21.62 ± 1.514[c]	0.003	<0.001	0.001
Relative areas	14.25 ± 1.870[a]	20.97 ± 1.691[ab]	21.48 ± 2.191[b]	22.73 ± 1.752[ab]	24.86 ± 1.865[c]	0.002	<0.001	0.001

CHO/L = carbohydrate/lipid.
[a-c] Values in the same row with different letter superscripts are significantly different ($P < 0.05$). Values are presented as mean ± SE ($n = 6$).

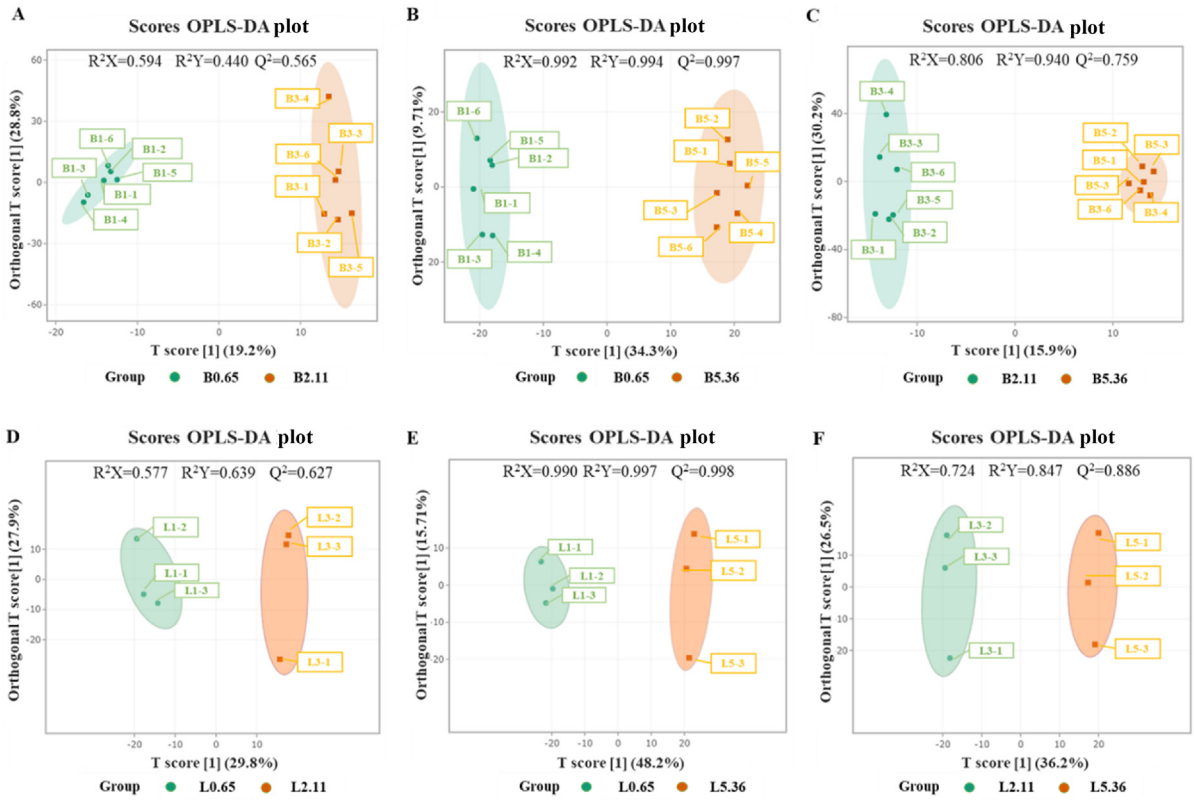

Fig. 3. Analysis of significantly differential metabolites in lipidomics analysis of broodstock ovarian (A, B, C) and 3 DPH larvae (D, E, F). Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) with corresponding cross-validation of metabolite profiles in B0.65 vs. B2.11 (A), B0.65 vs. B5.36 (B), and B2.11 vs. B5.36 (C), L0.65 vs. L2.11 (D), L0.65 vs. L5.36 (E), and L2.11 vs. L5.36 (F), respectively. B0.65, B2.11, and B5.36 represent broodstock ovarian with diet CHO/L ratios of 0.65, 2.11, and 5.36, while L0.65, L2.11, and L5.36 represent 3 DPH larvae with diet CHO/L ratios of 0.65, 2.11, and 5.36, respectively. DPH = day post-hatching.

analysis demonstrated a strong clustering pattern among the broodstock ovary groups (B0.65 vs. B2.11, B0.65 vs. B5.36, and B2.11 vs. B5.36) as well as the offspring larval groups (L0.65 vs. L2.11, L0.65 vs. L5.36, and L2.11 vs. L5.36), indicating clear distinctions between these groups (Fig. 3A–F). The results demonstrate the high predictability of the OPLS-DA-derived model, making it appropriate for all subsequent data analyses.

The OPLS-DA model was employed to facilitate the categorization and identification of differential lipid metabolites (DLM) among the groups. The number and composition of the lipid categories and subclasses detected in all samples are presented in Figs. S1–A and Table S1. A total of 1182 lipids were identified in ovary and offspring larvae samples, belonging to 6 lipid classes and 38 subclasses, containing triglycerides (TG), phosphatidyl ethanolamines (PE), phosphatidylcholines (PC), diacylglycerols (DG), carnitines (CAR), lyso-phosphatidylcholines (LPC), ceramides (Cert), phosphatidylinositol (PI), Sphingomyelin (SM), lyso-phosphatidylethanolamines (LPE), free fatty acid (FFA), phosphatidylserine (PS), phosphatidylglycerol (PG), lyso-N-acyl-PE (LNAPE), hexosylceramides (HexCer), cholesteryl esters (CE) and other lipid species. TG accounted for 26.1%, followed by PE and PC, which accounted for approximately 16.2% and 11.2%, respectively, whereas the other lipid subclasses exhibited lower proportions. Based on the Venn diagram (Fig. S1), 196 DLM were identified in the ovary samples. Specifically, there were 57 DLM found in the comparison between the B0.65 vs B2.11 groups, 173 DLM in the comparison between B0.65 vs B5.36 groups, and 52 DLM in the comparison between B2.11 vs B5.36 groups. A total of 217 DLM were detected in offspring larvae, including 35 DLM between the L0.65 vs L2.11 groups, 204 DLM between the L0.65 vs L5.36 groups, and 56 DLM the between L2.11 vs L5. There were 22 DLM common to all three parental groups and six DLM specific to the offspring. Compared with the B0.65 group, the differential metabolites in the B2.11 and B5.36 parental groups were successively downregulated. In addition to the upregulation of phosphatidyl ethanolamine-plasmalogens (PE-P), other differential lipid metabolites were downregulated in the offspring, as shown in Table S1.

3.6. Identification of DLM in the ovary and offspring larvae

The DLM were identified based on the following criteria: fold change (FC) ≥ 2.0 or ≤0.5 and VIP ≥1. Volcano plots depict the differential distribution of lipid metabolites without considering variable importance of projection. As shown in Fig. 4. There were 41 metabolites downregulated between the B0.65 vs B2.11 groups (Fig. 4A), 152 metabolites were downregulated and 20 metabolites were upregulated between B0.65 vs B5.36 (Fig. 4B), 38 metabolites were downregulated, and three metabolites were upregulated in the B2.11 vs B5.36 group (Fig. 4C). Meanwhile, 11 metabolites were downregulated and 7 metabolites were upregulated between the L0.65 vs L2.11 groups (Fig. 4D), 125 metabolites were downregulated and 53 metabolites were upregulated between the L0.65 vs L5.36 groups (Fig. 4E), and 19 metabolites were downregulated and 6 metabolites were upregulated in the L2.11 vs L5.36 group (Fig. 4F), respectively. The number of DLM in the B0.65 vs B5.36 comparison group was much more than that in the B0.56 vs B2.11 and B2.11 vs B5.36 group.

Lipidomic analysis was conducted to determine the relative content of DLM in the ovaries and larvae from the CHO/L0.65, CHO/L2.11 and CHO/L5.36 groups. Ovarian lipid classes are composed of five major categories: glycerolipids (TG, DG, and monoglyceride [MG]), fatty acyl (CAR and FFA), cholesterol ester (CE), glycerophospholipids (PC, phosphatidylethanolamine [PE], LPC, PI, LPE, PG and PS) and sphingolipids (SM and Cert) (Table 8). Interestingly, no sphingolipid metabolites were detected in the larvae (Table 9). As shown in Table 8, the content of the ovarian lipid metabolites TG, MG, CAR, FFA, PC, PI, and PG decreased significantly ($P < 0.05$), whereas the content of PE, LPC, LPE, and PS increased significantly with an increase in dietary CHO/L ratios ($P < 0.05$). The MG and Cert contents reached a maximum when the CHO/L ratio was 2.11. However, the ovarian CE content was not significantly different among the groups. The content of fatty acids (FFA and CAR) in the larvae showed the same trend as that in the ovaries. The contents of the metabolites DG, MG, PE, LPC, PI, LPE, and PS in larvae significantly increased with an increase in dietary CHO/L ratios ($P < 0.05$), whereas TG, PC, PG, and CE showed the opposite trend ($P < 0.05$) (Table 9).

3.7. Metabolic pathway of DLM in the ovary and offspring larvae

To investigate the potential metabolic pathways in the ovary and offspring larvae in response to varying dietary CHO/L ratios, the detected DLM were analyzed in relation to the metabolic pathways found in the KEGG database. As shown in Fig. 5A–F, the predominant metabolic pathways of differential metabolites among the parental ovarian were triglyceride metabolism, regulation of adipocyte lipolysis, vitamin digestion and absorption, lipid and atherosclerosis, cholesterol metabolism, fat digestion and absorption, insulin resistance, thermogenesis and other metabolic pathways (Fig. 5A–C). In addition, the differential metabolites between the B0.65 vs B5.36 groups (Fig. 5B) were linked to phosphatidylinositol metabolism, phosphatidylinositol signaling, and long-term inhibition pathways. Similarly, the differential metabolites of the L0.65 vs L5.36 (Fig. 5E) and L2.11 vs L5.36 groups (Fig. 5F) were mainly enriched in vitamin digestion and absorption, thermogenesis, regulation of adipocyte lipolysis, lipid and atherosclerosis, insulin resistance, triglyceride metabolism, fat digestion and absorption, and cholesterol metabolism. However, the L0.65 vs L2.11 group was mainly enriched in the thermogenic pathway (Fig. 5D).

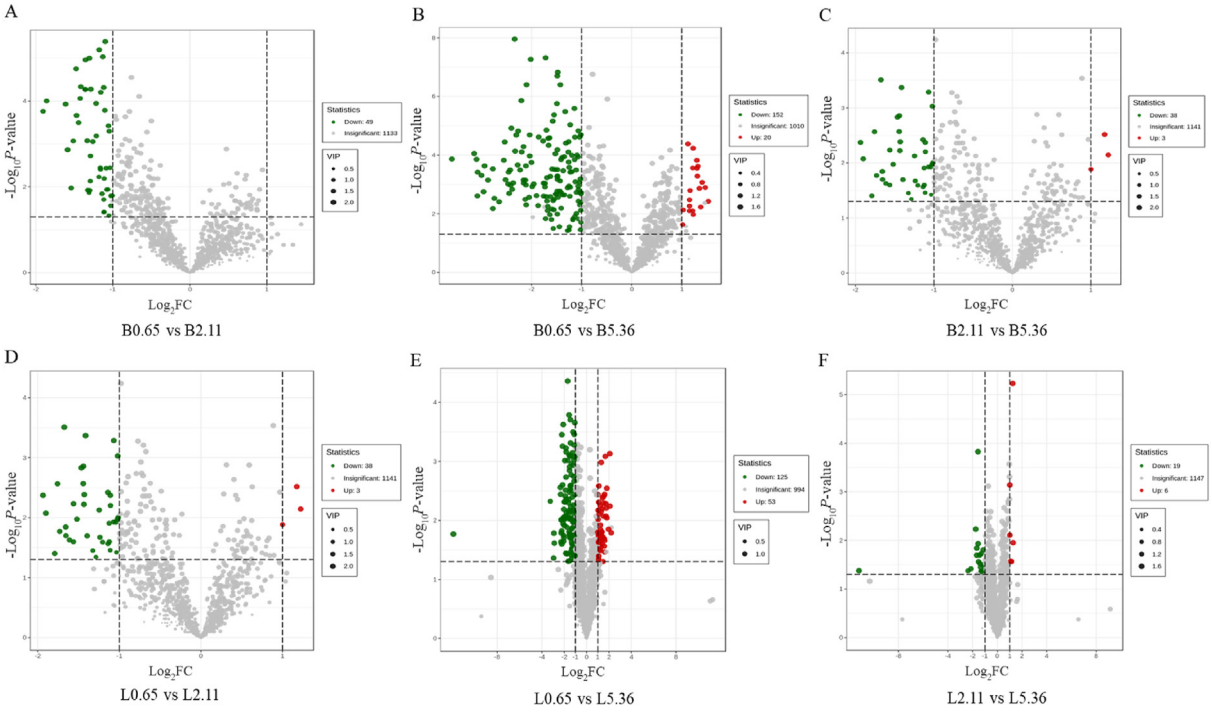

Fig. 4. Analysis of significantly differential metabolites in lipidomics analysis of broodstock ovarian (A, B, C) and 3 DPH larvae (D, E, F). Volcano plot analysis of B0.65 vs. B2.11 (A), B0.65 vs. B5.36 (B), and B2.11 vs. B5.36 (C), L0.65 vs. L2.11 (D), L0.65 vs. L5.36 (E), and L2.11 vs. L5.36 (F), respectively. B0.65, B2.11, and B5.36 represent broodstock ovarian with diet CHO/L ratios of 0.65, 2.11, and 5.36, while L0.65, L2.11, and L5.36 represent 3 DPH larvae with diet CHO/L ratios of 0.65, 2.11, and 5.36, respectively. The green points indicate significant down regulated metabolites between the two groups (FC ≤ 0.5 or ≥2.0; $P < 0.05$). The gray points show tentatively matched features with no significance. The red points indicate significant up regulated metabolites between the two groups. The horizontal coordinate represents the log value (Log_2 FC) of the multiple difference in relative content of a metabolite between the two groups of samples. The greater the absolute value of the horizontal coordinate, the greater the difference in relative content of a metabolite between the two groups of samples. VIP = variable importance in the projection; FC = fold change; DPH = day post-hatching.

Table 8
Relative contents of lipid classes in ovary of yellow catfish fed with diets containing 0.65, 2.11 and 5.36 of CHO/L ratios.

Lipid class	Lipid subclass	Dietary CHO/L ratios			*P*-value		
		0.65	2.11	5.36	ANOVA	Linear	Quadratic
Glycerolipid	TG ($\times10^6$)	862.89 ± 50.360^{c}	527.12 ± 50.971^{b}	306.18 ± 19.370^{a}	<0.001	<0.001	<0.001
	DG ($\times10^6$)	76.77 ± 8.171^{c}	51.24 ± 2.071^{b}	27.20 ± 0.590^{a}	<0.001	<0.001	0.001
	MG ($\times10^4$)	0.77 ± 0.071^{a}	1.80 ± 0.527^{b}	1.35 ± 0.071^{ab}	<0.001	0.371	0.272
Fatty acyl	FFA ($\times10^6$)	0.41 ± 0.052^{c}	0.31 ± 0.051^{b}	0.21 ± 0.074^{a}	<0.001	0.003	0.016
	CAR ($\times10^6$)	1.98 ± 0.172^{c}	1.42 ± 0.311^{b}	0.78 ± 0.064^{a}	<0.001	0.001	0.003
Glycerophospholipids	PC ($\times10^6$)	307.08 ± 11.714^{c}	218.51 ± 15.450^{b}	166.75 ± 10.970^{a}	<0.001	<0.001	<0.001
	PE ($\times10^6$)	12.99 ± 2.010^{a}	20.48 ± 2.921^{b}	27.87 ± 2.292^{c}	<0.001	<0.001	0.002
	LPC ($\times10^6$)	66.21 ± 6.620^{b}	73.50 ± 6.532^{b}	12.33 ± 7.433^{a}	<0.001	<0.001	<0.001
	PI ($\times10^6$)	9.98 ± 1.131^{b}	6.04 ± 1.143^{ab}	3.99 ± 0.484^{a}	<0.001	<0.001	0.002
	LPE ($\times10^4$)	46.68 ± 5.474^{a}	52.35 ± 5.463^{a}	102.55 ± 10.640^{b}	<0.001	<0.001	<0.001
	PG ($\times10^4$)	27.16 ± 4.714^{b}	16.90 ± 3.858^{ab}	11.73 ± 0.610^{a}	0.009	0.006	0.022
	PS ($\times10^4$)	9.25 ± 0.740^{a}	19.28 ± 5.330^{ab}	24.01 ± 2.110^{b}	0.025	0.006	0.021
Sphingolipid	SM ($\times10^4$)	313.04 ± 59.720^{a}	544.46 ± 99.604^{b}	639.49 ± 75.611^{b}	0.015	0.012	0.037
	Cert ($\times10^4$)	2.40 ± 0.581^{a}	4.98 ± 0.961^{b}	2.81 ± 0.361^{a}	0.023	0.732	0.038
Cholesterol ester	CE ($\times10^4$)	6.49 ± 0.990	6.89 ± 0.841	7.25 ± 1.040	0.064	0.574	0.858

CHO/L = carbohydrate/lipid; TG = triglyceride; DG = diglyceride; MG = monoglyceride; FFA = free fatty acid; CAR = acylcarnitines; PC = phosphatidylcholine; PE = phosphatidylethanolamine; LPC = lyso-phosphatidylcholine; PI = phosphatidylinositol; LPE = lyso-phosphatidylethanolamine; PG = phosphatidylglycerol; PS = phosphatidylserine; SM = sphingomyelin; Cert = ceramide; CE = cholesterol ester.
[a-c] Means in the same row with different lowercase have significantly different levels of lipid metabolites between ovaries ($P < 0.05$). Values are presented as mean ± SE ($n = 6$).

Table 9
Relative contents of lipid classes in 3 DPH larvae of yellow catfish fed with diets containing 0.65, 2.11 and 5.36 of CHO/L ratios.

Lipid class	Lipid subclass	Dietary CHO/L ratios			*P*-value		
		0.65	2.11	5.36	ANOVA	Linear	Quadratic
Glycerolipid	TG ($\times10^6$)	253.86 ± 12.610^{c}	168.77 ± 25.953^{b}	101.42 ± 11.141^{a}	<0.001	<0.001	0.003
	DG ($\times10^6$)	1.64 ± 0.110^{a}	2.01 ± 0.133^{ab}	2.27 ± 0.121^{b}	<0.001	0.005	0.027
	MG ($\times10^4$)	0.11 ± 0.031^{a}	0.18 ± 0.035^{ab}	0.24 ± 0.021^{b}	<0.001	0.019	0.080
Fatty acyl	FFA ($\times10^6$)	10.58 ± 0.671^{b}	8.32 ± 0.783^{ab}	6.39 ± 0.222^{a}	0.015	0.011	0.005
	CAR ($\times10^6$)	6.33 ± 0.222^{c}	3.56 ± 0.195^{b}	2.71 ± 0.163^{a}	<0.001	0.001	0.017
Glycerophospholipids	PC ($\times10^6$)	248.01 ± 17.552^{c}	228.89 ± 20.160^{b}	190.07 ± 6.850^{a}	<0.001	0.031	0.105
	PE ($\times10^6$)	4.68 ± 1.192^{a}	6.15 ± 0.251^{b}	10.94 ± 0.200^{c}	<0.001	0.001	0.001
	LPC ($\times10^6$)	11.57 ± 1.571^{a}	19.02 ± 2.951^{b}	26.44 ± 1.032^{c}	<0.001	0.001	0.006
	PI ($\times10^6$)	1.21 ± 0.260^{a}	2.19 ± 0.242^{b}	2.97 ± 0.131^{c}	<0.001	<0.001	0.004
	LPE ($\times10^4$)	20.90 ± 4.360^{a}	31.72 ± 3.413^{b}	50.47 ± 4.414^{c}	<0.001	0.001	0.006
	PG ($\times10^4$)	10.21 ± 1.113^{c}	6.26 ± 0.781^{b}	2.45 ± 0.334^{a}	<0.001	<0.001	0.002
	PS ($\times10^4$)	9.29 ± 1.883^{a}	17.13 ± 1.564^{b}	27.73 ± 0.470^{c}	<0.001	<0.001	<0.001
Cholesterol ester	CE ($\times10^4$)	107.02 ± 7.433^{c}	65.46 ± 6.990^{b}	51.76 ± 4.291^{a}	<0.001	0.001	0.002

CHO/L = carbohydrate/lipid; DPH = day post-hatching; TG = triglyceride; DG = diglyceride; MG = monoglyceride; FFA = free fatty acid; CAR = acylcarnitines; PC = phosphatidylcholine; PE = phosphatidylethanolamine; LPC = lyso-phosphatidylcholine; PI = phosphatidylinositol; LPE = lyso-phosphatidylethanolamine; PG = phosphatidylglycerol; PS = phosphatidylserine; CE = cholesterol ester; DPH = day post-hatching.
[a–c] Means in the same row with different lowercase letter superscripts have significantly different levels of lipid metabolites between 3 DPH larvae ($P < 0.05$). Values are presented as mean ± SE ($n = 3$).

To explore the metabolic pathways involved in maternal fat deposition and offspring health of yellow catfish fed different CHO/L diets, and to determine the correlation between metabolites and metabolic pathways in the maternal ovary and offspring larvae of yellow catfish fed three groups of different CHO/L diets were analyzed. The results indicated that the main metabolic pathways involved in ovarian (Fig. 5G) differential metabolites were glycerophospholipid metabolism, glycerolipid metabolism, sphingolipid metabolism, and the phosphatidylinositol signaling system, whereas the metabolic pathways of offspring larvae (Fig. 5H) were not involved in sphingolipid metabolism.

3.8. Ovary and offspring larvae mRNA levels of lipid metabolism-related genes

The relative mRNA levels of genes related to lipid metabolism in the maternal ovary and larvae were significantly influenced by the varying dietary CHO/L ratios, as illustrated in Fig. 6. In the ovarian tissue, the relative mRNA expression levels of *srebp1*, *acc*, *atgl*, *fad2* and *elvol5* were significantly upregulated with an increase in the dietary CHO/L ratio ($P_L < 0.05$). The relative mRNA expression of the *hsl* gene was significantly higher at a dietary CHO/L ratio of 2.11 than that at dietary CHO/L ratios of 0.65 and 1.44 ($P = 0.041$) (Fig. 6A–C). Conversely, the relative mRNA expression levels of *cpt* showed the opposite trend. In the 3 DPH larvae, the relative mRNA expression levels of genes involved in lipid synthesis (*srebp1*), lipolysis (*ppar*, *cpt* and *atgl*), and long chain polyunsaturated fatty acid synthesis (*fad2* and *elvol5*) initially increased and then decreased with increasing dietary CHO/L ratio ($P_Q < 0.05$), reaching a maximum at a CHO/L ratio of 2.11. However, the relative mRNA expression levels of *fas*, *acc* and *hsl* were not significantly different among the treatments (Fig. 6D–F).

4. Discussion

The growth performance of maternal yellow catfish decreased significantly when the dietary CHO/L ratio exceeded 2.11. Previous studies have also shown that the growth performance and feed utilization of various fish species, such as hybrid Clarias catfish (*Clarias microcephalus* × *C. gariepinus*) (Jantrarotai et al., 1994),

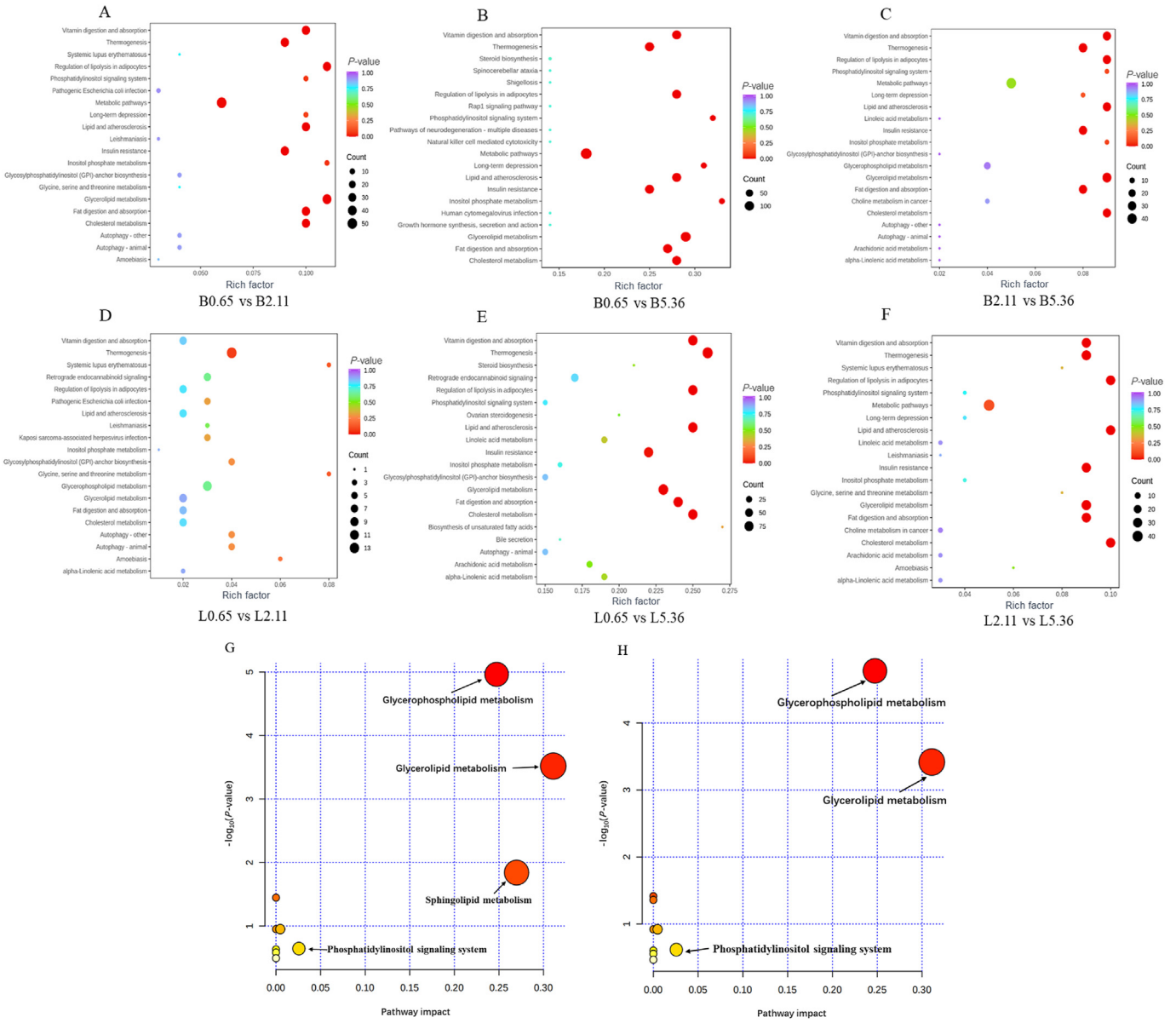

Fig. 5. KEGG enrichment bubble diagram of differential lipid metabolites in broodstock ovarian (A, B, C) and 3 DPH larvae (D, E, F). A bubble diagram of the differential metabolites of B0.65 vs. B2.11 (A), B0.65 vs. B5.36 (B), and B2.11 vs. B5.36 (C),L0.65 vs. L2.11 (D), L0.65 vs. L5.36 (E), and L2.11 vs. L5.36 (F), respectively. B0.65, B2.11, and B5.36 represent broodstock ovarian with diet CHO/L ratios of 0.65, 2.11, and 5.36, while L0.65, L2.11, and L5.36 represent 3 DPH larvae with diet CHO/L ratios of 0.65, 2.11, and 5.36, respectively. Differential metabolite enrichment pathway analysis of ovary (G) and 3 DPH larvae (H) of yellow catfish. The abscissa in the bubble graphs represents the rich factor (the greater the value, the greater the degree of enrichment), while the ordinate was the name of the passage. A deeper red color of the points indicates that the enrichment was more significant. The size of the spots represents the number of enriched differential metabolites. DPH = day post-hatching

Chinese longsnout catfish (*Leiocassis longirostris*) (Tan et al., 2007), and rockfish (*Sebastes schlegeli*) (Lee and Kim, 2009), show a similar pattern of initial increase followed by a decrease with increasing dietary CHO/L ratios. Interestingly, the optimal CHO/L ratio for maternal yellow catfish in this study was lower than that for juvenile yellow catfish (CHO/L ratio: 3.67) (Wang et al., 2014), and juvenile yellow catfish (*Pelteobagrus vachelli*) (CHO/L ratio: 4.06) (Zhang et al., 2011), suggesting that maternal yellow catfish may need more lipids as an energy source in preparation for reproduction, which is consistent with our previous findings (Fei et al., 2023). However, the dietary CHO/L ratio of 3.13 and 5.36 with crude lipid levels of 8.5% and 6.12%, respectively, did not meet the lipid requirements of maternal yellow catfish when the lipid level was below 9%, resulting in reduced reproductive performance. Variations in the optimal CHO/L ratio among different fish species may be influenced by factors such as culture environment, species characteristics, growth stage, feed formulation, and processing techniques.

The GSI serves as a crucial indicator of gonadal development. In this study, the GSI peaked at CHO/L ratios of 1.44 and 2.11, highlighting the positive impact of an appropriate CHO/L ratio on promoting gonadal development in yellow catfish. Maternal fertilization and hatching rates increased notably at dietary CHO/L ratios of 0.65 and 2.11. Conversely, a CHO/L ratio of 5.36 led to reduced egg diameter, decreased fecundity, higher malformation rates in post-hatching larvae, and smaller body length in 3 DPH larvae. These results suggest that a high-fat, low-carbohydrate diet can significantly enhance the reproductive performance of female

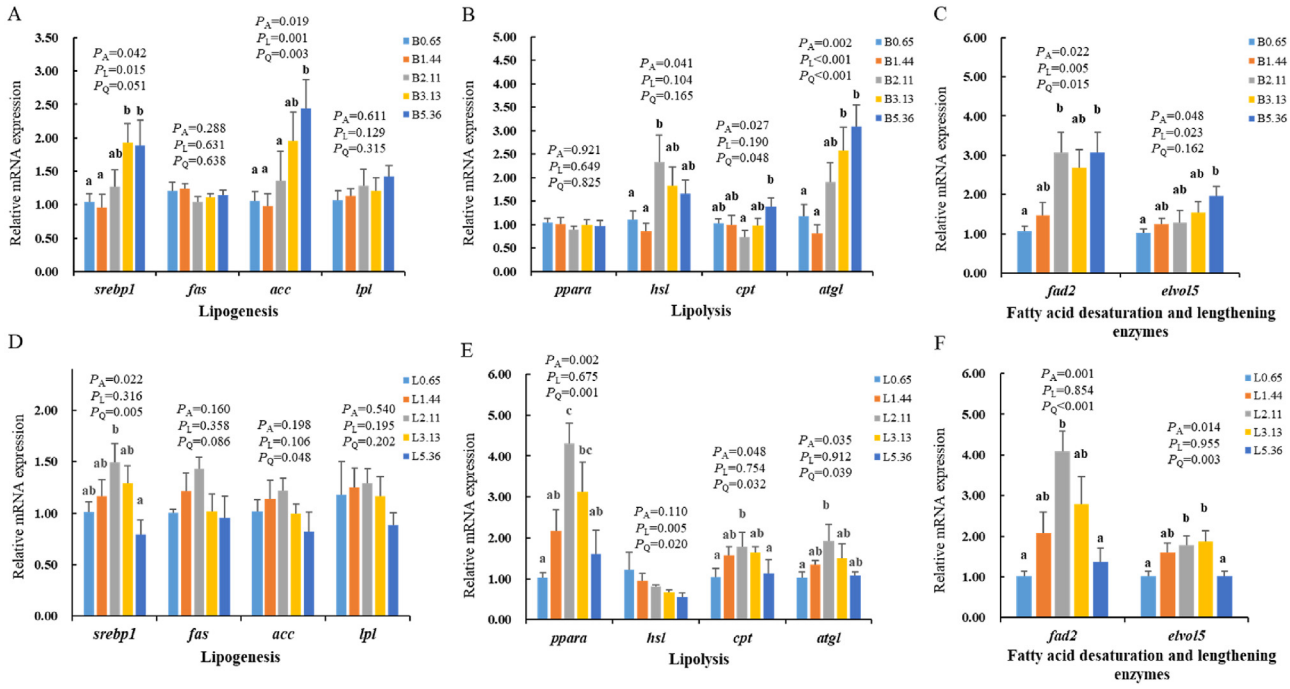

Fig. 6. Effects of dietary CHO/L ratio on expression of lipid metabolism related genes in broodstock ovarian (A, B, C) and 3 DPH larvae (D, E, F) of female yellow catfish. (A) The relative mRNA expression levels of lipogenesis genes in the ovary. (B) The relative mRNA expression levels of lipolysis genes in the ovary. (C) The relative mRNA expression levels of fatty acid desaturation and lengthening enzyme genes in the ovary. (D) The relative mRNA expression levels of lipogenesis genes in 3 DPH larvae. (E) The relative mRNA expression levels of lipolysis genes in 3 DPH larvae. (F) The relative mRNA expression levels of fatty acid desaturation and lengthening enzyme genes in the 3 DPH larvae. B0.65, B1.44, B2.11, B3.13, and B5.36 represent broodstock ovarian with diet CHO/L ratios of 0.65, 1.44, 2.11, 3.13 and 5.36, while L0.65, L1.44, L2.11, L3.13 and L5.36 represent 3 DPH larvae with diet CHO/L ratios of 0.65, 1.44, 2.11, 3.13 and 5.36, respectively. $^{a-c}$ Bars with different letters are significantly different ($P < 0.05$). Data are shown as mean ± SE ($n = 6$). P_A = *P*-value of ANOVA analysis; P_L = *P*-value of linear analysis; P_Q = *P*-value of quadratic analysis; DPH = day post-hatching; *fas* = fatty acid synthase; *srebp1* = sterol regulatory element binding protein 1; *acc* = acetyl-CoA carboxylase; *lpl* = lipoprotein *lipase; pparα* = peroxisome proliferator activated receptor *α*; *cpt* = carnitine palmitoyl transferase 1 isoform; *hsl* = hormone-sensitive lipase; *atgl* = adipose triglyceride lipase; *elvol5* = elongation of very long chain fatty acids protein 5; *fad2* = delta (12)-oleate desaturase.

yellow catfish compared to a high-carbohydrate, low-fat diet. Previous research has also demonstrated that increased fat intake can stimulate ovarian development, increase egg size, and improve fecundity (Kabir et al., 2019; Sink and Lochmann, 2008). During vitellogenesis, E2 concentration gradually increases as the gonads mature, stimulating the hepatic synthesis of VTG to support oocyte development (Hiramatsu et al., 2006). Testosterone is an androgen that plays a vital role in reproductive activities and serves as a precursor for estradiol synthesis in vivo (Miura and Miura, 2003). In this study, when the dietary CHO/L ratio ranged from 0.65 to 2.11, plasma levels of T and E2 remained consistently high but decreased significantly beyond a CHO/L ratio of 2.11. These results indicated that dietary CHO/L ratio between 0.65 and 2.11 effectively promotes ovarian development and steroid hormone secretion. Fish egg yolk is primarily derived from VTG, providing essential endogenous nutrition for larvae before they start feeding, underscoring the importance of both the quantity and quality of egg yolk to ensure the early survival, growth, and development of fish (Hiramatsu et al., 2015). This study revealed that a high-carbohydrate, low-fat diet consumed by yellow catfish mothers during the breeding season led to a significantly lower plasma VTG content. This change was consistent with the concurrent reduction in egg diameter, larval body length, and larval survival rate, implying that this dietary alteration could negatively affect reproductive performance and egg quality.

Additionally, an increase in the dietary CHO/L ratio initially enhanced ovarian lipid content, but was followed by a decline, whereas liver lipid deposition increased significantly. These results suggest that maintaining an appropriate dietary CHO/L ratio facilitates ovarian lipid accumulation and provides sufficient energy for oocyte development. Nevertheless, prolonged intake of a high-carbohydrate and low-fat diet may lead to excessive hepatic lipid accumulation, affecting maternal health and potentially impairing larval offspring. In agreement with previous studies in mice, livers from mice fed a high-carbohydrate diet showed more potent lipogenic and inflammatory effects than those from mice fed a high-fat diet, mainly by promoting the accumulation of saturated and monounsaturated fatty acids in the liver (Da Silva-Santi et al., 2016). Similar observations have been reported in studies on blunt snout bream (*Megalobrama amblycephala*) and largemouth bass (*Micropterus salmoides*) (Gong et al., 2022; Li et al., 2012). Previous studies have shown that an increase in the CHO/L ratio has no significant effect on the crude protein, moisture, or ash content of the whole body but significantly decreases the crude lipid content (Ren et al., 2021). However, in this study, the lipid content of the whole body was not affected by the CHO/L ratio of the diet, possibly because of increased energy consumption for reproduction and development during the breeding period in females.

Numerous studies have confirmed the crucial role of lipids in maternal biological processes, such as cell survival, differentiation, multiplication, and interaction, which are closely linked to the development and health of offspring (Rao et al., 2012; Smedts et al., 2012). In the present study, TG, DG, PE, and PC were the lipid subclasses with the largest number of DLM, and their abundance significantly decreased with increasing dietary CHO/L ratios. Notably, offspring larvae exhibited higher levels of FFA, with the CHO/L0.65 group showing significantly higher levels than the CHO/L5.36 group, indicating that a high-fat diet may lead to FFA deposition in offspring tissues. Studies have shown that as FFA accumulate, triglycerides may be ectopically deposited in the liver and

muscle where they can be converted into lipid intermediates, potentially impairing cellular function and causing lipotoxicity (Hayakawa et al., 2018). Inconsistent with the results of this study, TG, PC, and PE have been identified as the primary lipid metabolites in the muscle tissue of Nile tilapia (*Oreochromis niloticus* L.) (Liu et al., 2019) and the hepatopancreas of swimming crabs (*Portunus trituberculatus*) (Yuan et al., 2021) fed with different lipid sources. These results indicated that the distribution patterns of distinct lipid molecules vary across species and tissues.

The pathways of glycerophospholipid and glycerolipid metabolism showed significant enrichment among the differential metabolites between broodstock and offspring. Additionally, sphingolipid metabolism pathways were specifically enriched in the maternal ovaries, suggesting regulation by the dietary CHO/L ratio. Glycerophospholipids play a crucial role as structural components of biological membranes and act as messengers for signal regulation (Yang et al., 2019). In this study, the relative abundance of LPC, LPE, and PS in the ovary and offspring larvae significantly increased with an increase in the dietary CHO/L ratio, indicating that a maternal diet with high carbohydrate and low fat may specifically accumulate these lipid compounds. However, further investigation is required to elucidate their biological functions in vivo. In mammals, disruption of the glycerophospholipid pathway is crucial for the development of hematopoietic toxicity induced by benzene through its impact on autophagy. Additionally, the sphingolipid pathway may play a significant role in benzene-induced toxicity by regulating cellular proliferation and apoptosis (Yu et al., 2021). Sphingolipids are structurally similar to glycerolipids, as components of membrane structures and signaling molecules, have been reported to cause obesity or dyslipidemia (Hannun and Obeid, 2018). In addition, the relative abundance of SM increased with increasing CHO/L ratio in the ovary, whereas a significantly higher relative abundance of Cert was detected at a CHO/L ratio of 2.11. Cert contains a long-chain amino-alcohol sphingosine and a fatty acid, and elevated levels of ceramide are often associated with insulin resistance (Hannun and Obeid, 2018; Meikle and Summers, 2017). Cert serves as an effector molecule that plays a role in various downstream protein intermediates and functions as a regulator of membrane structure and tumor suppressor lipids. These functions typically lead to apoptosis, growth arrest, cell migration, adhesion, aging, and differentiation (Zeidan and Hannun, 2007). The findings of this study indicate that an appropriate dietary CHO/L ratio may promote the maintenance of ovarian cell homeostasis, which further indicates that a good parental nutritional history has potential benefits in improving the quality of offspring. This suggests that altering the dietary CHO/L ratio significantly affects the lipid composition of both ovaries and larvae of yellow catfish, potentially influencing cellular metabolic processes and functions.

Lipid deposition is a complex process that involves lipid uptake, transport, decomposition, and anabolic processes. The utilization of lipids and carbohydrates by fish is well documented. Lipid metabolism is regulated by ingested carbohydrates and glucose (Azaza et al., 2015; Li et al., 2016). In the present study, the transcription levels of the fat-synthesizing gene *lpl* in the ovaries and larvae of yellow catfish were not affected by the dietary CHO/L ratio. Similarly, the liver *lpl* gene expression levels in large yellow croaker (*Larimichthys crocea*) fed diets with different fat levels were also unaffected (Yan et al., 2015). However, the relative expression of key genes regulating fat synthesis (*srebp1* and *acc*) and polyunsaturated fatty acid synthesis (*fad2* and *elvol5*) in the ovary was significantly upregulated with an increase in dietary CHO/L ratio. This implies that excessive carbohydrate intake increases endogenous fat synthesis. Intriguingly, genes associated with lipid metabolism initially showed upregulation, followed by downregulation as the CHO/L ratio increased in the larvae. This suggests that an appropriate CHO/L ratio can effectively maintain adipogenesis and catabolic homeostasis in the offspring, thus preserving their healthy physiological state. Previous studies on fish have shown that low-fat intake or excessive carbohydrate consumption can upregulate liver lipogenesis-related genes and downregulate lipolysis-related genes (Li et al., 2016; Xiong et al., 2014). Therefore, a high carbohydrate and low fat diet may enhance lipid deposition by increasing de novo fat synthesis and reducing β-oxidation of fatty acids.

5. Conclusion

Optimal growth and egg quality in maternal yellow catfish were achieved with a CHO/L ratio between 1.91 and 2.11, along with dietary lipid levels of 9.4% to 10.3% and carbohydrate levels of 20.8% to 21.8%. During the reproductive period, broodfish showed better tolerance to a high-fat diet than to a high-carbohydrate diet. A high CHO/L diet led to increased lipogenesis and impaired reproductive performance and offspring quality. Glycerophospholipid metabolism, glycerolipid metabolism, and the phosphatidylinositol signaling system were identified as the key metabolic pathways associated with lipid metabolism in the ovaries and larvae exposed to diets with different CHO/L ratios. Interestingly, sphingolipid metabolism was not found to be a significant pathway in larvae. However, when the dietary CHO/L ratio exceeded 3.13, the dietary lipid level failed to meet the lipid requirement of female yellow catfish. Therefore, as a limitation of this study, it is necessary to further study the dietary CHO/L ratio of the broodstock under the minimum lipid requirement.

Credit author Statement

Shuzhan Fei: Data curation, Investigation, Methodology, Writing – Original draft. **Zheng Chen:** Data curation, Investigation, Methodology. **Haokun Liu:** Conceptualization, Software. **Junyan Jin:** Conceptualization, Validation. **Yunxia Yang:** Investigation, Methodology. **Dong Han:** Conceptualization, Writing – Review & Editing. **Xiaoming Zhu:** Conceptualization, Investigation, Resources, Writing – Review & Editing. **Shuoqi Xie:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgements

This research was supported by the National Key Research and Development Program of China (2022YFD2400900), the earmarked fund for China Agriculture Research System (CARS-46). We would like to thank Dr. Yuanhui Duan for assisting with farming and sample collection of experimental fish.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.07.003.

References

AOAC. Official methods of analysis. 18th ed. Gaithersburg, MD: AOAC International; 2006.

Azaza MS, Khiari N, Dhraief MN, Aloui N, Kraem MM, Elfeki A. Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. Aquacult Res 2015;46(1):14–27.

Chou R, Su MS, Chen HY. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 2001;193(1):81–9.

Da Silva-Santi LG, Antunes MM, Caparroz-Assef SM, et al. Liver fatty acid composition and inflammation in mice fed with high-carbohydrate diet or high-fat diet. Nutrients 2016;8(11):682.

FBMA, Fisheries Bureau of Ministry of Agriculture. China fishery statistics yearbook. Beijing, China: China Agriculture Press; 2023.

Fei SZ, Chen Z, Duan YH, Liu HK, Jin JY, Yang YX, et al. Growth, reproduction, fatty acid profiles and offspring performance of broodstock yellow catfish *Pelteobagrus fulvidraco* fed diets with different lipid levels. Aquaculture 2023;580:740273.

Gong YL, Lu QS, Liu YL, Xi LW, Zhang ZM, Liu HK, et al. Dietary berberine alleviates high carbohydrate diet-induced intestinal damages and improves lipid metabolism in largemouth bass (*Micropterus salmoides*). Front Nutr 2022;9:1010859.

Hannun YA, Obeid LM. Sphingolipids and their metabolism in physiology and disease. Nat Rev Mol Cell Biol 2018;19(3):175–91.

Hayakawa J, Wang M, Wang CY, Han RH, Jiang ZY, Han XL. Lipidomic analysis reveals significant lipogenesis and accumulation of lipotoxic components in ob/ob mouse organs. Prostaglandins Leukot Essent Fatty Acids 2018;136:161–9.

Hiramatsu N, Matsubara A, Fujita T, Sullivan CV, Hara A. Multiple piscine vitellogenins: biomarkers of fish exposure to estrogenic endocrine disruptors in aquatic environments. Mar Biol 2006;149(1):35–47.

Hiramatsu N, Todo T, Sullivan CV, Schilling J, Reading BJ, Matsubara T. Ovarian yolk formation in fishes: molecular mechanisms underlying formation of lipid droplets and vitellogenin-derived yolk proteins. Gen Comp Endocrinol 2015;221:9–15.

Huang Y, Ye T, Liu C, Fang F, Chen Y, Dong Y. Maternal high-fat diet during pregnancy and lactation affects hepatic lipid metabolism in early life of offspring rat. J Biosciences 2017;42:311–9.

Izquierdo M, Fernandez-Palacios H, Tacon A. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture 2001;197(1–4):25–42.

Jantrarotai W, Sitasit P, Rajchapakdee S. The optimum carbohydrate to lipid ratio in hybrid Clarias catfish (*Clarias macrocephalus*×*C. gariepinus*) diets containing raw broken rice. Aquaculture 1994;127(1):61–8.

Kabir MA, Munir MB, Wan Alias SLF, Leja A, Lee SW, Hashim R. Effect of different dietary lipid levels on spawning performance and egg quality of pangasianodon hypophthalmus (Sauvage, 1878). Asian Fish Sci 2019;32(4).

Lee SM, Kim KD. Effects of dietary carbohydrate to lipid ratios on growth and body composition of juvenile and grower rockfish, *Sebastes schlegeli*. Aquacult Res 2009;40(16):1830–7.

Li XS, Zhu XM, Han D, Yang YX, Jin JY, Xie SQ. Carbohydrate utilization by herbivorous and omnivorous freshwater fish species: a comparative study on gibel carp (*Carassius auratus gibelio*. var CAS III) and grass carp (*Ctenopharyngodon idellus*). Aquacult Res 2016;47(1):128–39.

Li XF, Liu WB, Lu KL, Xu WN, Ying W. Dietary carbohydrate/lipid ratios affect stress, oxidative status and non-specific immune responses of fingerling blunt snout bream, *Megalobrama amblycephala*. Fish Shellfish Immunol 2012;33(2):316–23.

Lin SM, Shi CM, Mu MM, Chen YJ, Luo L. Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, *Micropterus salmoides*. Fish Shellfish Immunol 2018;78:121–6.

Liu Y, Jiao JG, Gao S, Ning LJ, McHele Limbu S, Qiao F, et al. Dietary oils modify lipid molecules and nutritional value of fillet in Nile tilapia: a deep lipidomics analysis. Food Chem 2019;277:515–23.

Lu KL, Xu WN, Wang LN, Zhang DD, Zhang CN, Liu WB. Hepatic β-oxidation and regulation of carnitine palmitoyltransferase (CPT) I in blunt snout bream *Megalobrama amblycephala* fed a high fat diet. PLoS One 2014;9(3):e93135.

Luo Z, Liu YJ, Mai KS, Tian LX, Liu DH, Tan XY, et al. Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper *Epinephelus coioides* juveniles fed isonitrogenous diets in floating netcages. Aquacult Int 2005;13(3):257–69.

Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. Nat Rev Endocrinol 2017;13(2):79–91.

Miura T, Miura CI. Molecular control mechanisms of fish spermatogenesis. Fish Physiol Biochem 2003;28(1–4):181–6.

NRC. Nutrient requirements of fish and shrimp. Washington, DC: National Academies Press; 2011. p. 102–25.

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001;29(9):e45.

Quiclet C, Dubouchaud H, Berthon P, Sanchez H, Vial G, Siti F, Fontaine E, Batandier C, Couturier K. Maternal exercise modifies body composition and energy substrates handling in male offspring fed a high-fat/high-sucrose diet. J Physiol 2017;595(23):7049–62.

Rao KR, Padmavathi I, Raghunath M. Maternal micronutrient restriction programs the body adiposity, adipocyte function and lipid metabolism in offspring: a review. Rev Endocr Metab Disord 2012;13:103–8.

Ren MC, Ai QH, Mai KS, Ma HM, Wang XJ. Effect of dietary carbohydrate level on growth performance, body composition, apparent digestibility coefficient and digestive enzyme activities of juvenile cobia, *Rachycentron canadum L.* Aquac. Res. 2011;42(10):1467–75.

Ren MC, Liang HL, Ge XP, Chen XR, Ji K. Effects of dietary carbohydrate-to-lipid ratio on growth performance, plasma parameters and hepatic antioxidant status in ide (*Leuciscus idus*, Linnaeus, 1758). Aquac. Rep. 2021;19(4):1–9.

Sink TD, Lochmann RT. Effects of dietary lipid source and concentration on channel catfish (*Ictalurus punctatus*) egg biochemical composition, egg and fry production, and egg and fry quality. Aquaculture 2008;283(1–4):68–76.

Smedts H, van Uitert EM, Valkenburg O, Laven J, Eijkemans M, Lindemans J, et al. A derangement of the maternal lipid profile is associated with an elevated risk of congenital heart disease in the offspring. Nutr Metabol Cardiovasc Dis 2012;22(6):477–85.

Tan Q, Xie S, Zhu X, Lei W, Yang Y. Effect of dietary carbohydrate-to-lipid ratios on growth and feed utilization in Chinese longsnout catfish (*Leiocassis longirostris Günther*). J Appl Ichthyol 2007;23(5):605–10.

Wang LN, Liu WB, Lu KL, Xu WN, Cai DS, Zhang CN. Effects of dietary carbohydrate/lipid ratios on non-specific immune responses, oxidative status and liver histology of juvenile yellow catfish *Pelteobagrus fulvidraco*. Aquaculture 2014;426(2):41–8.

Wenk MR. The emerging field of lipidomics. Nat Rev Drug Discov 2005;4(7):594–610.

Xiong YY, Huang JF, Li XX, Zhou LL, Dong F, Ye H. Deep sequencing of the tilapia (*Oreochromis niloticus*) liver transcriptome response to dietary protein to starch ratio. Aquaculture 2014;433:299–306.

Yan J, Liao K, Wang TJ, Mai KS, Xu W, Ai QH. Dietary lipid levels influence lipid deposition in the liver of large yellow croaker (*Larimichthys crocea*) by regulating lipoprotein receptors, fatty acid uptake and triacylglycerol synthesis and catabolism at the transcriptional level. PLoS One 2015;10(6):1–15.

Yang F, Liu MY, Qin NK, et al. Lipidomics coupled with pathway analysis characterizes serum metabolic changes in response to potassium oxonate induced hyperuricemic rats. Lipids Health Dis 2019;18:1–10.

Yu L, Sun R, Xu K, et al. Lipidomic analysis reveals disturbances in glycerophospholipid and sphingolipid metabolic pathways in benzene-exposed mice. Toxicol Res 2021;10(4):706–18.

Yuan Y, Xu FM, Jin M, Wang XX, Hu XY, Zhao MM. Untargeted lipidomics reveals metabolic responses to different dietary n-3 PUFA in juvenile swimming crab (*Portunus trituberculatus*). Food Chem 2021;354:129570.

Zeidan YH, Hannun YA. Translational aspects of sphingolipid metabolism. Trends Mol Med 2007;13(8):327–36.

Zhou C, Liu B, Ge X, Xie J, Xu P. 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 Appl Ichthyol 2013;29(6):1348–56.

Zhou PP, Wang MQ, Xie FJ, Deng DF, Zhou QC. Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker (*Larmichthys crocea*). Aquaculture 2016;453(4):45–51.