

Effects of dietary carbohydrate and lipid levels on growth performance, hepatic histology and antioxidant capacity, and flesh texture of mandarin fish (*Siniperca chuatsi*)

Zhihao Han^{a,1}, Ye Gong^{a,1}, Nihe Zhang^a, Zeqiang Sun^a, Shengchao Liu^a, Xuxiong Huang^{a,b}, Naisong Chen^a, Songlin Li^{a,b,*}

^aResearch Centre of the Ministry of Agriculture and Rural Affairs on Environmental Ecology and Fish Nutrition, Shanghai Ocean University, Shanghai, 201306, China

^bNational Demonstration Center on Experiment Teaching of Fisheries Science, Shanghai Ocean University, Shanghai, 201306, China

***Corresponding author: Songlin Li**, Shanghai Ocean University, Shanghai, 201306, China, Tel./Fax: +86 21 61900465, mail address: slli@shou.edu.cn

¹These authors contributed equally to this work.

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Abstract

In this study, nine isonitrogenous experimental diets containing graded level of carbohydrates (40 g/kg, 80 g/kg and 120 g/kg) and crude lipid (80 g/kg, 120 g/kg, and 160 g/kg) were formulated in a two-factor (3×3) orthogonal design. 945 mandarin fish with similar body weight were randomly assigned into 27 tanks and the experiment diets were fed to triplicate tanks twice daily for 10 weeks. Results showed that different dietary treatments did not significantly affect survival rate and growth performance of mandarin fish. However, high dietary lipid and carbohydrate level significantly decreased the protein content of whole body and muscle of cultured fish. The lipid content of whole body, liver and muscle all significantly increased with increasing levels of dietary lipid, while only liver lipid level was significantly affected by dietary carbohydrate level. Hepatic glycogen content increased significantly with increasing dietary carbohydrate level. As to liver antioxidant capacity, malondialdehyde content increased significantly with increasing dietary lipid or carbohydrate content and catalase activity showed an opposite trend. Superoxide dismutase activity increased significantly with increasing levels of dietary lipid but decreased first and then increased with increasing dietary carbohydrate level. Additionally, the increase of both dietary lipid and carbohydrate levels resulted in a significant reduction in muscle hardness. Muscle chewiness, gumminess and shear force were only affected by dietary lipid levels and decreased significantly with increasing dietary lipid levels. In conclusion, considering all the results, the appropriate dietary lipids and carbohydrates level for mandarin fish were 120 g/kg and 80 g/kg, respectively.

Abbreviations: ANOVA, analysis of variance; CAT, catalase; CF, condition factor; FBW, final body weight; FER, feed efficiency rate; FI, feed intake; HSI, hepatosomatic index; MDA, malondialdehyde; PER, protein efficiency rate; SGR, specific growth rate; SOD, superoxide dismutase; SR, survival rate; T-AOC, total antioxidative capacity; TP, total protein; TPA, texture profiles analysis; VSI, viscerosomatic index.

Introduction

Mandarin fish (*Siniperca chuatsi*) is highly favored among Chinese, just as described in the “A Fisherman's Song” authored by the poet, Zhihe Zhang, in Tang Dynasty. In 2023, the production of mandarin fish was above 470,000 tons⁽¹⁾. However, the mandarin fish largely rely on live bait and in practice, about less than 10% of the total production of compound feed have been applied in the cultivation of this fish species⁽²⁾, which mainly due to the lack of studies on its nutrient requirement.. Hence, studies relating to the nutritional physiological characteristics of this fish species could produce significant beneficial to its cultivation sector.

Carbohydrates and lipid act as main non-protein energy sources of aquafeed, and their potential protein-sparing effects have been well documented in teleosts^(3,4). Carbohydrates is the cheapest energy source and acts as a binder in aquafeed. However, considerable studies have proved that dietary carbohydrates content for most warm water carnivorous fish should be less than 100 g/kg, and excessive dietary carbohydrate could lead to metabolic disorders, which further induce the reduction in growth and feed utilization, suppression of immune function and increased pathogens susceptibility to cultured fish, such as the study on largemouth bass (*Micropterus salmoides*)⁽⁵⁾, hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂)⁽⁶⁾ and red-spotted grouper (*Epinephelus akaara*)⁽⁷⁾. Lipid is another essential energy source, which are also important sources of essential fatty acids and function as the carriers of fat-soluble vitamins⁽⁴⁾. However, excessive dietary lipid also produces negative impacts on farmed fish, such as abnormal lipid deposition, oxidative stress, and inflammation response, as reported in large yellow croaker (*Larimichthys crocea*)⁽⁸⁾, Japanese seabass (*Lateolabrax japonicus*)⁽⁹⁾ and turbot (*Scophthalmus maximus* L.)⁽¹⁰⁾. Therefore, suitable dietary carbohydrates and lipids content for carnivorous fish should be determined, while it cannot be truly defined due to the interaction between dietary lipid and carbohydrate as described in some fish species, such as gilthead sea bream (*Sparus aurata*)⁽¹¹⁾, hybrid grouper⁽¹²⁾ and large yellow croaker⁽¹³⁾. However, to date, little information is available on the interaction between dietary carbohydrate and lipid in mandarin fish.

In recent years, with the improvement of living standard, the flesh quality of farmed fish attracting more attention. In practice, some consumers believe that the flesh quality of farmed mandarin fish fed compound feed do not match those fed forage fish. It has been well documented that dietary compositions affect the flesh quality, including nutritional and sensory quality of farmed fish⁽¹⁴⁾. The texture and structure of fish muscle are important fresh quality attributes, which include hardness, springiness, chewiness, cohesiveness, resilience,

and internal cross-linking of connective tissue⁽¹⁵⁾. Growing evidences have confirmed that dietary lipid content is a basis for the forming of flesh texture, and muscle lipid deposition commonly soften fish flesh⁽¹⁶⁻¹⁸⁾. Meanwhile, the variation in dietary carbohydrate content leads to glycogen deposition and influences the flesh texture as reported in farmed dentex (*Dentex dentex*)⁽¹⁹⁾ and olive flounder (*Paralichthys olivaceus*)⁽²⁰⁾. Therefore, it's suggested that dietary carbohydrate and lipid level and their interactions might also affect the flesh quality of mandarin fish.

Considering all above, a two-factor (3×3) orthogonal study was designed to investigate the effects of dietary carbohydrates and lipid level on growth performance, hepatic histology and antioxidant capacity and flesh quality of mandarin fish. It is envisaged that present study would be helpful to enrich the current knowledge of this fish species and further promotes the development of this aquaculture sector.

Materials and methods

Experimental diets

Nine isonitrogenous (crude protein 520 g/kg) experimental diets containing graded level of carbohydrates (40 g/kg, 80 g/kg and 120 g/kg) and crude lipid (80 g/kg, 120 g/kg, and 160 g/kg) were formulated in a 3 × 3 factorial design (Table 1). The feed ingredients were mixed thoroughly and then produced a stiff dough following the description in Li et al⁽⁶⁾. Then, the dough was extruded with a pelleting machine through the 2.5 mm, 3.5 mm and 6.0 mm die, respectively, to form different particle sizes with the length of 1.3 cm, 1.7 cm and 2.5 cm, respectively. After that, the pellets were cooked for starch gelatinization (105 °C, 15 min) and dried (55 °C, 6 h) in a ventilated oven. Then, the diets were stored at -20 °C until the experiment being started.

Experiment procedure

The feeding trial was conducted in the joint laboratory of Shanghai Ocean University and Guangdong Evergreen Feed Industry Co., Ltd (Zhanjiang, China). The juvenile mandarin fish, average body weight of 15 g, were obtained from Guangdong Liangshi Aquatic Seed Industry Co., Ltd (Guangzhou, China), which were then domesticated using the experimental diet (120 g/kg crude lipid, 40 g/kg carbohydrate). After four weeks' acclimation, total 945 fish with similar body weight (55 g) were randomly assigned into 27 tanks (35 fish/tank; water volume, 1000 L). Triplicate tanks of fish were fed one of the experimental diets twice daily (7:00 and

16:30) for 10 weeks. During the feeding trial, the fish were gradually fed the diet with particle diameter of 2.5 mm (body weight < 50 g), 3.5 mm (body weight < 120 g) and 6.0 mm (body weight > 120 g). The feeding trial was conducted in a circulating water system, which was equipped with a thermostat and a UV lamp for disinfection. During the experiment process, siphon was used to remove the dirt from the bottom of the bucket and discharge the sewage from the filtration tank two hour after feeding, and then groundwater was added to the system as a supplement. The water quality of the system was monitored to be consistent throughout the entire feeding process. Water quality variables were maintained at pH 8.0 ± 0.2 , temperature 28 ± 0.5 °C, nitrite content less than or equal to 0.01 mg/L, ammonia nitrogen less than 0.2 mg/L, and nitrate nitrogen within 25 to 50 mg/L.

Sample Collection

After 10 weeks' feeding trial, the total fish number and fish weight were recorded after the fish being fasted for 24 h and the fish for sampling were anesthetized with eugenol (1:10000). Then, fifteen fish were randomly selected for further sample collection. Briefly, for each tank, five fish were randomly collected for the analysis of the composition of whole body, and the individual body length and weight of the remaining ten fish were measured for the calculation of condition factor (CF). Then, the liver and visceral mass of six fish were separated for hepatosomatic index (HSI) and viscerosomatic index (VSI) calculation, respectively. After that, liver samples were collected for histological analysis, proximate composition analysis and enzyme activity analysis, and muscle samples were separated for the analysis of proximate composition and texture.

Body proximate composition analysis

Body composition was analyzed based on the method in AOAC⁽²¹⁾. Moisture content was measured by drying samples to a constant weight (# 934.01; AOAC, 2003), ash content was determined by combustion (# 942.05; AOAC, 2003) and Kjeldahl nitrogen determination method was used to measure crude protein content (KD310, Opsi, Sweden) (# 976.05; AOAC, 2003). Soxhlet extraction method was taken to measure crude lipid content (SX-360, Opsi, Sweden) (# 920.29; AOAC, 2003). The potassium hydroxide/anthrone was used to measure hepatic glycogen content⁽²²⁾, following a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Hepatic antioxidant capacity analysis

The liver tissue samples were cleaned with precooled physiological saline at 4 °C, and then the surface water of the liver sample was absorbed using absorbent paper. The cleaned liver samples were mixed with phosphate buffer and then homogenised using a tissue homogeniser (1:9, w/v, 10,000-15,000 r/min) and centrifuged (2000 rpm, 10 min, 4°C) to separate the supernatants. The total antioxidative capacity (T-AOC), malondialdehyde (MDA) content and the activity of superoxide dismutase (SOD) and catalase (CAT) were analyzed using commercial kits following the instruction (Nanjing Jiancheng Bioengineering Institute, Nanjing, China; Catalogue Number: TP, A045-2-2; MDA, A003-1; T-AOC, A015-2-1; CAT, A007-1-1; SOD, A001-3). In detail, total antioxidant capacity (T-AOC) was evaluated by measuring the content of Fe^{2+} reduced from Fe^{3+} , which could form a solid orange-red complex with phenanthroline analogs, and its antioxidant capacity could be measured by colorimetry⁽²³⁾. The total superoxide dismutase (T-SOD) activity was measured with the ferricytochrome C method⁽²⁴⁾. Malondialdehyde (MDA), a degradation product of lipid peroxidation, condenses with thiobarbituric acid to form a red product with a maximum absorption peak at 532 nm⁽²⁵⁾. The above indices were expressed as specific activity, and the soluble protein content was determined with the coomassie brilliant blue method⁽²⁶⁾. Catalase (CAT) activity was determined by measuring the decreased concentration of H_2O_2 per minute according to Aebi⁽²⁷⁾.

Hepatic histology analysis

After sampling, liver samples were cut into 1 cm³ and fixing with 4% paraformaldehyde (PFA). Then the sample were dehydrated and embedded in paraffin wax. After that, the samples were sectioned at 5- μm -thickness for subsequent staining. The slides were deparaffinized and rehydrated with dimethyl benzene and graded concentrations of ethanol stained with hematoxylin and eosin in conjunction. Finally, the images were captured (40 \times) the Olympus-DP73 optical microscope (Olympus, Tokyo, Japan) was used to capture images.

Flesh quality analysis

The texture analysis of the dorsal muscle was performed with texture profiles analysis (TPA) using a Universal TA device (TA. ZM-Plus, DEXIN Technology (Kunming) Co., Ltd., China). Test conditions were set as previously described by Xu et al⁽²⁸⁾.

Statistical methods

All data were analyzed using SPSS version 22. The interaction effects of dietary lipid and carbohydrate levels on cultured fish were analyzed by factorial (two-way) analysis of variance (ANOVA). If the interaction was not significant, the Tukey's multiple range test was followed to determine the effects of single main effect (lipid or carbohydrate). If the interaction was significant, one-way ANOVA followed by Tukey's multiple range tests were used to determine the effects of one factor under different levels of another factor. The significance level was set at $P < 0.05$.

Result

Growth performance and feed utilization

Different dietary lipid and carbohydrate levels did not significantly affect survival rate (SR), final body weight (FBW), specific growth rate (SGR), feed intake (FI), feed efficiency rate (FER) and protein efficiency rate (PER) of mandarin fish ($P > 0.05$) (Table 2). The fish fed dietary lipid (160 g/kg) and carbohydrate level (120 g/kg) displayed significantly higher condition factor (CF) and viscerosomatic index (VSI) compared to those fed low dietary lipid level (80 g/kg) and low carbohydrate level (40 g/kg), respectively, ($P < 0.05$) (Table 2). Hepatosomatic index (HSI) was only affected by dietary carbohydrate level and significantly increased with increasing carbohydrate levels ($P < 0.05$) (Table 2).

Approximate body composition

Whole body and liver moisture content of mandarin fish decreased significantly with increasing dietary lipid level ($P < 0.05$) (Table 3). No significant effects were observed on whole body moisture content with increasing carbohydrate level ($P > 0.05$) (Table 3). However, fish fed carbohydrate level of 40 g/kg displayed significantly higher liver moisture content to those fed 80 g/kg and 120 g/kg carbohydrate ($P < 0.05$) (Table 3). High dietary lipid and carbohydrate level significantly decreased the protein content of whole body and muscle of cultured fish ($P < 0.05$) (Table 3), however only dietary carbohydrate level affected liver protein content ($P < 0.05$) (Table 3). Additionally, significant interaction was observed between these two main factors in muscle protein content ($P < 0.05$) (Table 3). The lipid content of whole body, liver and muscle all significantly increased with increasing levels of lipid in the feed ($P < 0.05$) (Table 3), while only liver lipid level was significantly increased as the increase of dietary carbohydrate level ($P < 0.05$) (Table 3). Hepatic glycogen content increased significantly with increasing dietary carbohydrate levels ($P < 0.05$) (Table 3). However, among different dietary lipid level groups, hepatic glycogen content reached the

highest value in the fish feeding 120 g/kg dietary lipid ($P < 0.05$) (Table 3) and significant interaction between the main factors in hepatic glycogen content was observed ($P < 0.05$) (Table 3).

Liver antioxidant capacity

Significant interaction of two main factors in liver MDA content and SOD and CAT activities of cultured fish were observed ($P < 0.05$) (Table 4). In detail, MDA content was significantly different among the dietary treatments, with its highest content measured in fish fed 160 g/kg lipid and 120 g/kg carbohydrate levels ($P < 0.05$) (Table 4). Liver CAT activity decreased significantly with increasing dietary lipid or carbohydrate level ($P < 0.05$) (Table 4). Hepatic SOD activity increased significantly with increasing level of lipid content in the feed ($P < 0.05$) (Table 4) however decreased first and then increased with the increasing level of dietary carbohydrate ($P < 0.05$) (Table 4).

Liver morphology

As shown in Fig 1, when the dietary crude lipid level were at 80 g/kg and 120 g/kg, and the dietary carbohydrate level were at 40 g/kg and 80 g/kg, the result showed that the hepatocytes of mandarin fish were uniformly arranged and regular, and the cell morphology was relatively normal, with occasional small-volume lipid droplets appearing (Fig1. A, B, D, E). When the dietary carbohydrate level reached 120 g/kg, the hepatocytes size began to become larger, with blurred edges, and the cells appeared to be swollen and vacuolated and the nuclei of the cells were shifted (Fig1. C, F, I). At high lipid level (160 g/kg), compared with medium lipid level (120 g/kg) and low lipid level (80 g/kg), hepatocytes were surrounded by a large number of oval lipid droplets, with irregular cell morphology and obvious vacuolation (Fig1. G, H, D). At high carbohydrate level (120 g/kg), the interaction effect produced by the increase in the crude lipid level exacerbated the swelling and vacuolisation of the cells, resulting in the confusion of cellular arrangement, the increase volume of lipid droplets, the disappear of some cells nuclei and the necrosis of some hepatocyte tissues occurred (Fig1. F, I).

Flesh texture quality

Muscle hardness of cultured fish decreased significantly with increasing level of dietary lipid under different dietary carbohydrate levels ($P < 0.05$) (Table 5). However, with the

dietary carbohydrate changing, it only significantly decreased in fish feeding 120 g/kg carbohydrate compared with those feeding 40 g/kg and 80 g/kg carbohydrate ($P < 0.05$) (Table 5). Additionally, only at 120 g/kg and 160 g/kg dietary lipid levels, muscle hardness was significantly affected by dietary carbohydrate content ($P < 0.05$) (Table 5). The significant interaction between two factors was also observed in muscle resilience ($P < 0.05$) (Table 5). However, muscle chewiness, gumminess and shear force were only affected by dietary lipid level, which were significantly decreased with the increase of dietary lipid level ($P < 0.05$) (Table 5).

Discussion

The result of the present study showed that the survival, growth performance and feed utilization of cultured mandarin fish, including SR, SGR, PER and FER, were not significantly affected by different dietary lipid and carbohydrate levels. It is worth noting that the growth performance of cultured fish in the present study was comparable to the production practice, which was outstanding compared with some current studies on mandarin fish^(29, 30). This was possibly related to the fact that under the present culture situation, all the designed feed formulas could satisfy the growth requirement of cultured fish and the experiment diets had a high palatability.

Considerable research has demonstrated the protein-sparing effects of dietary lipid and carbohydrates in aquatic animals^(3, 4, 31-34). The growth of cultured fish in this study was not affected by dietary lipid levels (80 g/kg-160 g/kg), indicating that the lowest lipid level in this study could satisfy the lipid requirement of mandarin fish, which was similar to the lipid requirement of red spotted grouper⁽³⁵⁾, largescale shoveljaw fish (*Onychostoma macrolepis*)⁽³⁶⁾ and loach (*Paramisgurnus dabryanus*)⁽³⁷⁾. For carnivorous fish, limited glucose utilization ability have been well illustrated and the recommended dietary carbohydrate level should be no more than 200 g/kg⁽³⁸⁾. In the present study, the increasing carbohydrate level from 40 g/kg to 120 g/kg did not significant affect the survival, growth performance and feed utilization of cultured mandarin fish, which were in line with the study in Yangtze sturgeon (*Acipenser dabryanus*)⁽³⁹⁾ and hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂)⁽⁴⁰⁾. However, dietary carbohydrate level exceed 100 g/kg would negatively affect the growth performance of largemouth bass⁽⁴¹⁾ and hybrid grouper⁽⁶⁾, which might because mandarin fish had a higher carbohydrate tolerance and the underline mechanism required further exploration.

Although the growth performance and the feed utilization were not significantly affected by dietary lipid and carbohydrate levels, significant differences were observed in body compositions among different treatment groups. In the present study, liver and muscle crude lipid content of cultured mandarin fish showed an increase trend with the increasing level of dietary lipid and carbohydrate, which could also be confirmed by the H&E staining results. This observation was similar to the study on turbot⁽⁴²⁾, lumpfish (*Cyclopterus lumpus*)⁽⁴³⁾ and triploid rainbow trout (*Oncorhynchus mykiss*)⁽⁴⁴⁾. In addition, liver glycogen significantly increased with the increase of dietary carbohydrate level and the same trend was found under each dietary lipid levels, which was in line with the results of hybrid snakehead⁽⁴⁰⁾ and spotted sea bass (*Lateolabrax maculatus*)⁽⁴⁵⁾. Interestingly, liver glycogen was not affected by dietary lipid level at low carbohydrate level, however, when carbohydrate reached 120 g/kg, liver glycogen was higher in 120 g/kg and 160 g/kg lipid group than 80 g/kg lipid group, which might be because that at high dietary carbohydrate level, increasing dietary lipid could negatively affect liver glycometabolism of mandarin fish, but the underline mechanism required further investigation. Furthermore, high liver lipid and glycogen content might lead to liver injury^(8, 46). In the present study, the liver injury could be observed in high lipid and high carbohydrate groups. The increased dietary lipid and carbohydrate level also reduced muscle protein content and the increased dietary lipid level enhanced muscle lipid content, which indicated that dietary lipid and carbohydrate inclusion level would also affect muscle compositions and further affect muscle growth of mandarin fish⁽⁴⁷⁾.

Increased liver lipid and glycogen content would affect liver antioxidant capacity of aquatic animals^(48, 12). As a representative cell peroxidation product, MDA content could directly reflect peroxidation damage⁽⁴⁹⁾. In the present study, the MDA content increased with the increase of dietary lipid and carbohydrate level, which indicated the liver peroxidation damage induced by high dietary lipid and carbohydrate level. The existence of antioxidant enzymes, such as CAT and SOD could effectively reduce peroxidation damage, thus they play an essential role in balancing the oxidation and anti-oxidation defense systems^(50, 51). CAT could catalyze H₂O₂ decomposition and SOD could catalyze superoxide radical dismutation, both of which could reflect reactive oxygen species (ROS) removal ability⁽⁵²⁾. In the present study, liver CAT activity was significantly decreased with the increase of dietary lipid and carbohydrate level, which was similar to the study on largemouth bass⁽⁵³⁾, mud crab (*Scylla paramamosain*)⁽⁵⁴⁾ and spotted sea bass⁽⁴⁵⁾. However, the SOD activity significantly increased in both high lipid (160 g/kg) group and high carbohydrate (120 g/kg) group, which

seems to be conflict to previous studies. The main reason here might be that although 160 g/kg lipid level and 120 g/kg carbohydrate level might not be the suitable level for mandarin fish, its effect was within the limit of the fish adaptive capacity and the increased SOD activity indicated the self-regulation process, which might also be the reason that explained the unaffected growth performance of mandarin fish under different lipid and carbohydrate levels.

Since dietary lipid and carbohydrate level significantly affected muscle protein and lipid content, both of which were essential factors affecting flesh texture quality, the flesh texture quality indexes were further analyzed^(20, 55). In the present study, the muscle hardness and resilience decreased with the increase of dietary lipid and carbohydrate level, which might related to the interaction of dietary lipid and carbohydrate induced muscle protein content decrease and this result was in line with previous study on the relationship between muscle protein content and flesh texture⁽⁵⁶⁾. However, the chewiness, gumminess and shear force only react to dietary lipid changes, which might due to the relation between flesh quality and muscle lipid content^(57, 58).

In conclusion, dietary lipid and carbohydrate level up to 160 g/kg and 120 g/kg, respectively, would not significantly affect the growth performance and feed utilization of mandarin fish, but high lipid and carbohydrate level negatively affect liver antioxidant capacity and flesh quality of mandarin fish. Taking liver histology and antioxidant capacity, flesh texture quality and feed processing characteristics into account, the appropriate level of lipid and carbohydrate in feed of mandarin fish were 120 g/kg and 80 g/kg, respectively. This study provided reliable experimental data and theoretical basis for the application of artificial compound feed for mandarin fish.

Author contributions

Zhihao Han: Investigation, Data curation, Formal analysis, Writing-Review & Editing; **Ye Gong :** Conceptualization, Supervision, Writing-Original Draft; **Nihe Zhang:** Investigation, Methodology; **Zeqiang Sun:** Investigation, Methodology; **Shengchao Liu:** Investigation, Methodology; **Xuxiong Huang:** Conceptualization, Investigation; **Naisong Chen:** Conceptualization, Project administration, Funding acquisition; **Songlin Li:** Conceptualization, Supervision, Writing-Review & Editing.

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Ethic Approval

The experiment was conducted following the procedure of Animal Care and Use Committee of Shanghai Ocean University.

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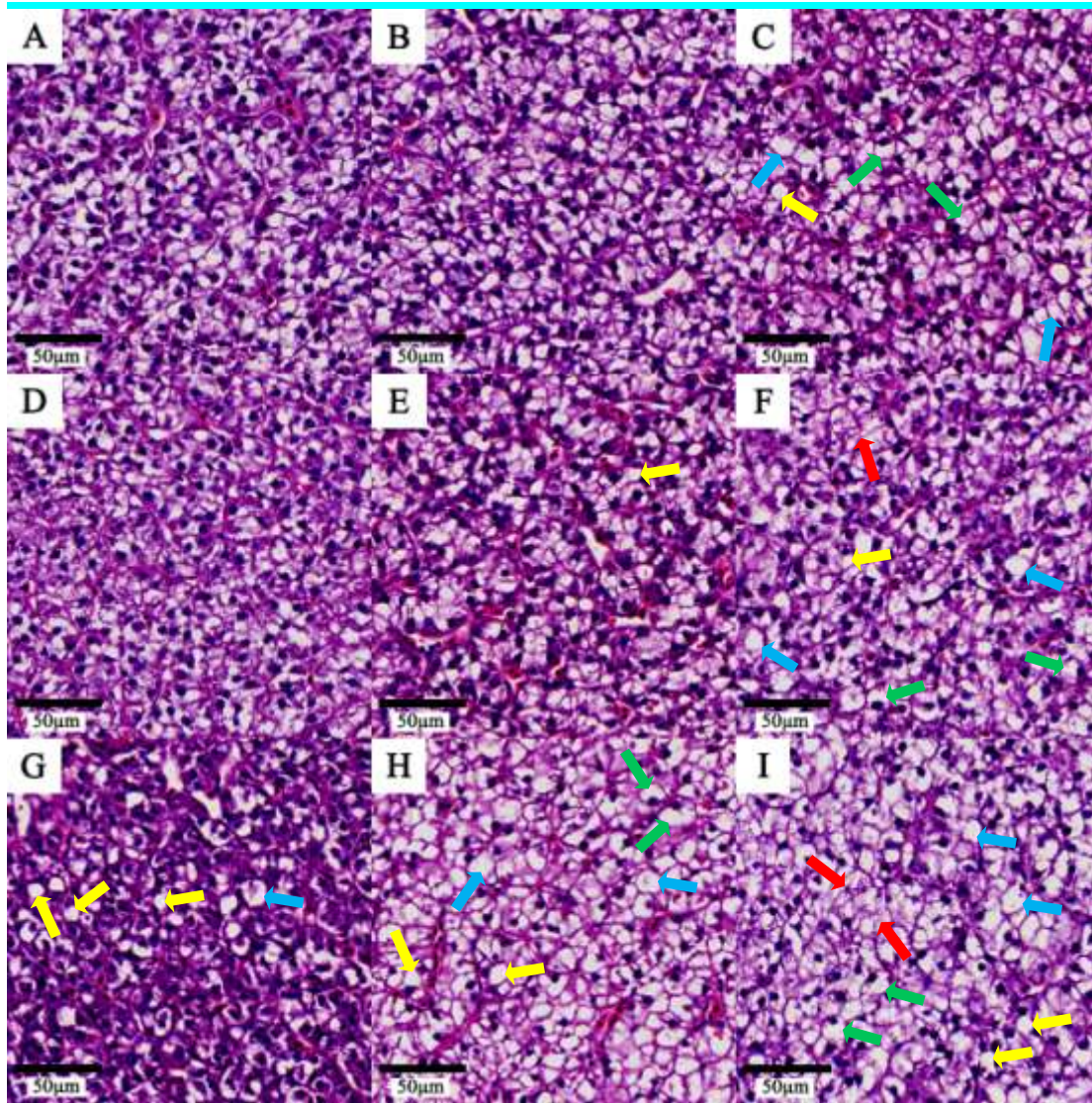
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Notes: A-C: 8% crude lipid, Carbohydrate level were 4%, 8%, 12%; D-F: 12% crude lipid, Carbohydrate level were 4%, 8%, 12%; G-I: 16% crude lipid, Carbohydrate level were 4%, 8%, 12%. LD: Lipid droplets (yellow arrow); SC: Swelling cells (green arrow); HPV: Hepatocyte vacuolation (blue arrow); FN: Focal necrosis (red arrow).

Fig1. Effects of dietary lipid and carbohydrate interactions on the histomorphology of the liver of mandarin fish (40 ×).

Table 1. Formulation and chemical composition of experimental diets (g/kg dry matter).

Carbohydrate (g/kg)	level	80 crude lipid			120 crude lipid			160 crude lipid		
		40	80	120	40	80	120	40	80	120
White fish meal ^a		560.0	560.0	560.0	560.0	560.0	560.0	560.0	560.0	560.0
Shrimp meal ^a		30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Wheat gluten meal ^a		40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Fermented meal ^a	soybean	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Blood cell powder ^a		27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5	27.5
Squid paste ^a		20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Beer yeast ^a		20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin mixture ^b		10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Mineral mixture ^c		12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Ca(H ₂ PO ₃) ₂ ^a		10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Phospholipid oil ^a		30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Soybean oil ^a		-	-	-	40.0	40.0	40.0	80.0	80.0	80.0
α -starch ^a		40.0	80.0	120.0	40.0	80.0	120.0	40.0	80.0	120.0
Microcrystalline cellulose		80.0	60.0	40.0	80.0	60.0	40.0	80.0	60.0	40.0
Zeolite powder ^a		80.0	60.0	40.0	80.0	60.0	40.0	80.0	60.0	40.0

Food attractant	0.5	0.5	0.5	00.5	00.5	00.5	00.5	00.5	00.5	
(DMPT)										
Proximate analysis (Mean values, g/kg dry weight)										
Moisture	32.0	34.0	36.0	30.0	32.0	38.0	35.0	35.0	36.0	
Crude protein	526.4	519.3	505.5	511.0	504.8	507.3	508.1	504.5	504.8	
Crude lipid	77.1	71.2	64.0	112.6	106.9	104.2	151.8	145.6	146.6	
Ash	191.0	187.0	182.0	190.0	186.0	182.0	186.0	182.0	180.0	
Starch	53.0	93.0	128.0	55.0	90.0	124.0	50.0	87.0	115.0	

^a Supplied by Xinxin Tian'en Aquatic Feed Co., Ltd (Zhejiang, China).

^b Vitamin Premix (mg/kg diet): vitamin A, 16000 IU; vitamin D₃, 8000 IU; vitamin K₃, 14.72; vitamin B₁, 17.80; vitamin B₂, 48; vitamin B₆, 29.52; vitamin B₁₂, 0.24; vitamin E, 160; vitamin C, 800; niacinamide, 79.20; calcium-pantothenate, 73.60; folic acid, 6.40; biotin, 0.64; inositol, 320; choline chloride, 1500; L-carnitine, 100.

^c Mineral Premix (mg/kg diet): Cu (CuSO₄), 2.00; Zn (ZnSO₄), 34.4; Mn (MnSO₄), 6.20; Fe (FeSO₄), 21.10; I (Ca (IO₃)₂), 1.63; Se (Na₂SeO₃), 0.18; Co (CoCl₂), 0.24; Mg (MgSO₄·H₂O), 52.7.

Table 2. Growth performance and feed utilization of mandarin fish fed diets with varying lipid and carbohydrate levels for 10 weeks.

Lipid (g/kg)	Carbohydrate (g/kg)	IBW (g)	FBW (g)	SR (%)	FI (g/fish/d)	SGR (%/day)	FER	PER	CF	VSI (%)	HSI (%)
Individual treatment means¹											
80	40	55.05	222.04	88.33	2.37	1.85	0.97	1.92	2.31	7.02	1.05
80	80	55.03	220.06	88.33	2.41	1.85	0.96	1.90	2.48	7.60	1.08
80	120	54.94	226.40	89.17	2.40	1.89	0.99	1.94	2.53	7.17	1.09
120	40	54.99	227.39	86.67	2.41	1.89	1.00	1.97	2.52	7.61	0.98
120	80	55.01	224.35	86.67	2.48	1.78	0.91	1.81	2.48	8.17	1.15
120	120	54.96	227.68	88.33	2.44	1.90	0.98	1.92	2.56	8.54	1.12
160	40	55.01	235.93	87.50	2.40	1.92	1.03	2.04	2.54	8.22	0.97
160	80	55.02	227.66	91.67	2.38	1.92	1.01	1.95	2.65	8.92	1.10
160	120	55.04	232.49	87.50	2.42	1.90	1.00	1.90	2.60	9.09	1.24
80	40	55.05	222.04	88.33	2.37	1.85	0.97	1.92	2.31	7.02	1.05
120	40	54.99	227.39	86.67	2.41	1.89	1.00	1.97	2.52	7.61	0.98
160	40	55.01	235.93	87.50	2.40	1.92	1.03	2.04	2.54	8.22	0.97
80	80	55.03	220.06	88.33	2.41	1.85	0.96	1.90	2.48	7.60	1.08
120	80	55.01	224.35	86.67	2.48	1.78	0.91	1.81	2.48	8.17	1.15
160	80	55.02	227.66	91.67	2.38	1.92	1.01	1.95	2.65	8.92	1.10
80	120	54.94	226.40	89.17	2.40	1.89	0.99	1.94	2.53	7.17	1.09
120	120	54.96	227.68	88.33	2.44	1.90	0.98	1.92	2.56	8.54	1.12
160	120	55.04	232.49	87.50	2.42	1.90	1.00	1.90	2.60	9.09	1.24
Means of main effect											
80		55.01	222.83	88.61	2.40	1.86	0.97	1.92	2.44 ^a	7.26 ^a	1.08
120		54.99	226.47	87.22	2.44	1.85	0.96	1.90	2.52 ^{ab}	8.11 ^b	1.08
160		55.02	232.03	88.75	2.40	1.92	1.01	1.96	2.60 ^b	8.74 ^c	1.10
	40	55.02	228.45	87.50	2.39	1.89	1.00	1.98	2.46 ^A	7.62 ^A	1.00 ^A
	80	55.02	224.02	88.89	2.42	1.85	0.96	1.89	2.53 ^A	8.23 ^B	1.11 ^B

B											
	120	54.98	228.86	88.33	2.42	1.90	0.99	1.92	2.56 ^B	8.27 ^B	1.15 ^B
Two-way ANOVA: P-values²											
	L	0.798	0.324	0.579	0.284	0.121	0.06	0.369	0.003	<	0.822
	C	0.780	0.675	0.712	0.613	0.264	0.25	0.161	0.043	0.003	0.004
	L × C	0.869	0.985	0.637	0.832	0.345	0.48	0.450	0.143	0.328	0.182

¹ Treatment means represent the average values of three tanks per treatment.

² Differences were regarded as significant when $P < 0.05$. L and C showed the main effect of each factor, and L × C indicated their interactive effect.

IBW, initial body weight; FBW, final body weight; SR, survival rate; FI, feed intake; SGR, specific growth rate; FER, feed efficiency ratio; PER, protein efficiency ratio; CF, condition factor; VSI, viscerosomatic index; HSI, hepatosomatic index.

Survival rate (SR, %) = final fish number / initial fish number × 100;

Feed intake (FI, g / fish / d) = dry feed consumption / [(final fish number + initial fish number) / 2] / experimental days;

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

Feed efficiency ratio (FER) = increased body weight / dry feed consumption;

Protein efficiency ratio (PER) = (final body weight – initial body weight) / protein intake;

Condition factor (CF, %) = final body weight (g) / length (cm)³ × 100;

Viscerosomatic index (VSI, %) = viscera weight / final body weight × 100;

Hepatosomatic index (HSI, %) = liver weight / final body weight × 100.

Table 3. Body composition of mandarin fish fed diets with varying lipid and carbohydrate levels for 10 weeks (g/100g wet weight).

Lipid (g/kg)	Carbohydrate (g/kg)	Whole body				Liver				Muscle		
		Moisture	Protein	Lipid	Ash	Moisture	Protein	Lipid	Glycogen	Moisture	Protein	Lipid
Individual treatment means¹												
80	40	74.70	17.07	3.42	4.36	74.13	14.10 ^a	3.76	3.45 ^a	79.34	18.99 ^c	0.87
80	80	74.80	17.04	3.15	4.41	73.39	14.86 ^b	4.01	5.56 ^b	80.33	18.09 ^b	0.83
80	120	75.09	16.37	3.23	4.23	74.52	14.60 ^a b	3.93	6.97 ^c	80.90	17.33 ^a	0.85
120	40	73.86	16.42	5.48	4.47	74.96	14.98	4.52	4.10 ^a	80.50	17.59 ^a	0.84
120	80	73.68	16.31	4.87	4.59	72.58	14.55	4.69	6.43 ^b	80.18	17.93 ^b	0.88
120	120	74.45	16.18	4.86	4.37	73.05	14.30	4.93	7.57 ^c	80.50	17.78 ^{ab}	0.86
160	40	73.64	16.16	5.50	4.37	73.92	14.94	5.44	3.53 ^a	80.15	16.81 ^a	1.02
160	80	73.93	15.84	6.09	4.04	72.41	14.78	6.03	5.36 ^b	80.72	17.33 ^b	0.92
160	120	73.67	15.51	6.63	3.99	71.61	13.74	6.17	7.65 ^c	81.00	17.03 ^{ab}	1.08
80	40	74.70	17.07	3.42	4.36	74.13	14.10 ^a	3.76	3.45 ^a	79.34	18.99 ^c	0.87
120	40	73.86	16.42	5.48	4.47	74.96	14.98 ^b	4.52	4.10 ^b	80.50	17.59 ^b	0.84
160	40	73.64	16.16	5.50	4.37	73.92	14.94 ^b	5.44	3.53 ^a	80.15	16.81 ^a	1.02
80	80	74.80	17.04	3.15	4.41	73.39	14.86	4.01	5.56 ^a	80.33	18.09 ^b	0.83
120	80	73.68	16.31	4.87	4.59	72.58	14.55	4.69	6.43 ^b	80.18	17.93 ^b	0.88
160	80	73.93	15.84	6.09	4.04	72.41	14.78	6.03	5.36 ^a	80.72	17.33 ^a	0.92
80	120	75.09	16.37	3.23	4.23	74.52	14.60	3.93	6.97 ^a	80.90	17.33 ^b	0.85
120	120	74.45	16.18	4.86	4.37	73.05	14.30	4.93	7.57 ^b	80.50	17.78 ^c	0.86
160	120	73.67	15.51	6.63	3.99	71.61	13.74	6.17	7.65 ^b	81.00	17.03 ^a	1.08
Means of main effect												
80		74.86 ^b	16.83 ^c	3.27 ^a	4.33	74.01 ^b	14.52	3.90 ^a	5.33 ^a	80.19	18.14 ^c	0.85 ^a

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b												
120		74.00 ^a	16.30 ^b	5.07 ^b	4.48	73.53 ^b	14.51	4.71 ^b	6.04 ^c	80.40	17.77 ^b	0.86 ^a
160		73.75 ^a	15.84 ^a	6.07 ^c	4.13 ^a	72.65 ^a	14.49	5.88 ^c	5.51 ^b	80.96	17.06 ^a	1.01 ^b
	40	74.07	16.55 _B	4.80	4.40	74.34 ^A	14.67 _A	4.57 ^A	3.70 ^A	80.33	17.80 ^B	0.91
	80	74.14	16.40 _B	4.70	4.35	72.79 ^B	14.73 _A	4.91 ^B	5.78 ^B	80.41	17.78 ^B	0.87
	120	74.40	16.02 _A	4.91	4.19	73.06 ^B	14.22 _B	5.01 ^B	7.40 ^C	80.80	17.38 ^A	0.93
Two-way ANOVA: P-values²												
	L	0.013	< 0.001	< 0.001	0.006	0.007	0.802	< 0.001	< 0.001	0.581	< 0.001	< 0.001
	C	0.613	< 0.001	0.686	0.102	0.002	0.023	0.028	< 0.001	0.801	< 0.001	0.156
	L × C	0.836	0.077	0.071	0.373	0.052	0.011	0.578	0.001	0.869	< 0.001	0.172

¹ Treatment means represent the average values of three tanks per treatment.

² Differences were regarded as significant when $P < 0.05$. L and C showed the main effect of each factor, and L × C indicated their interactive effect.

Table 4. Liver antioxidant capacity of mandarin fish fed diets with varying lipid and carbohydrate levels for 10 weeks.

Lipid (g/kg)	Carbohydrate (g/kg)	T-AOC (U/mgprot)	MDA (nmol/mgprot)	CAT (U/mgpro)	SOD (U/mgpro)
Individual treatment means¹					
80	40	0.08	0.28	699.96 ^c	449.60 ^b
80	80	0.07	0.28	680.62 ^b	382.59 ^a
80	120	0.07	0.30	581.54 ^a	496.30 ^c
120	40	0.08	0.27 ^a	638.29 ^c	431.09 ^a
120	80	0.07	0.29 ^b	575.64 ^a	444.08 ^b
120	120	0.06	0.32 ^c	589.15 ^b	472.29 ^c
160	40	0.07	0.29 ^a	570.60 ^c	502.11 ^b
160	80	0.07	0.30 ^a	509.11 ^b	500.72 ^b
160	120	0.06	0.32 ^b	475.06 ^a	490.44 ^a
80	40	0.08	0.28	699.96 ^c	449.60 ^b
120	40	0.08	0.27	638.29 ^b	431.09 ^a
160	40	0.07	0.29	570.60 ^a	502.11 ^c
80	80	0.07	0.28 ^a	680.62 ^c	382.59 ^a
120	80	0.07	0.29 ^b	575.64 ^b	444.08 ^b
160	80	0.07	0.30 ^b	509.11 ^a	500.72 ^c
80	120	0.07	0.30 ^a	581.54 ^b	496.30 ^c
120	120	0.06	0.32 ^b	589.15 ^c	472.29 ^a
160	120	0.06	0.32 ^b	475.06 ^a	490.44 ^b
Means of main effect					
80		0.07	0.28 ^a	654.04 ^c	442.83 ^a
120		0.07	0.29 ^b	601.02 ^b	449.15 ^b
160		0.07	0.30 ^c	518.26 ^a	497.76 ^c
	40	0.08	0.28 ^A	636.28 ^C	460.93 ^B
	80	0.07	0.29 ^B	588.45 ^B	442.46 ^A
	120	0.06	0.31 ^C	548.58 ^A	486.34 ^C
Two-way ANOVA: P-values²					
	L	-	< 0.001	< 0.001	< 0.001
	C	-	< 0.001	< 0.001	< 0.001
	L × C	-	< 0.001	< 0.001	< 0.001

T-AOC: total anti-oxidative capacity; MDA: malondialdehyde; CAT: catalase; SOD: superoxide dismutase.

¹ Treatment means represent the average values of three tanks per treatment.

² Differences were regarded as significant when $P < 0.05$. L and C showed the main effect of each factor, and L × C indicated their interactive effect.

Table 5. Flesh texture quality of mandarin fish fed diets with varying lipid and carbohydrate levels for 10 weeks.

Lipid (g/kg)	Carbohydrate (g/kg)	Hardness (gf)	Springiness	Chewiness (gf)	Resilience	Gumminess (gf)	Shear force (g.sec)
Individual treatment means¹							
80	40	671.17	0.49	174.06	0.45 ^b	352.63	2131.82
80	80	643.12	0.49	172.32	0.43 ^b	350.96	2402.62
80	120	643.63	0.49	165.35	0.39 ^a	335.64	2339.76
120	40	589.66 ^a	0.50	134.47	0.40	297.29	2009.62
120	80	629.13 ^b	0.48	166.33	0.42	343.60	1987.02
120	120	600.82 ^a	0.48	163.70	0.41	338.66	1721.58
160	40	516.69 ^b	0.49	129.61	0.39	264.85	1817.27
160	80	512.06 ^{ab}	0.49	135.62	0.41	276.98	1660.88
160	120	486.95 ^a	0.49	122.80	0.38	251.70	1387.91
80	40	671.17 ^c	0.49	174.06	0.45 ^b	352.63	2131.82
120	40	589.66 ^b	0.50	134.47	0.40 ^b	297.29	2009.62
160	40	516.69 ^a	0.49	129.61	0.39 ^a	264.85	1817.27
80	80	643.12 ^b	0.49	172.32	0.43	350.96	2402.62
120	80	629.13 ^b	0.48	166.33	0.42	343.60	1987.02
160	80	512.06 ^a	0.49	135.62	0.41	276.98	1660.88
80	120	643.63 ^c	0.49	165.35	0.39	335.64	2339.76
120	120	600.82 ^b	0.48	163.70	0.41	338.66	1721.58
160	120	486.95 ^a	0.49	122.80	0.38	251.70	1387.91
Means of main effect							
80		652.64 ^c	0.49	170.58 ^b	0.43 ^b	346.41 ^b	2291.40 ^b
120		606.53 ^b	0.49	154.90 ^b	0.41 ^a	326.52 ^b	1906.08 ^a
160		505.23 ^a	0.49	129.34 ^a	0.39 ^a	264.51 ^a	1622.02 ^a
	40	592.51 ^B	0.49	146.11	0.41 ^B	304.92	1986.24
	80	594.77 ^B	0.49	158.09	0.42 ^B	323.85	2016.84
	120	577.13 ^A	0.49	150.62	0.39 ^A	308.67	1816.42
Two-way ANOVA: P-values²							
	L	< 0.001	0.721	< 0.001	0.001	< 0.001	0.001
	C	0.012	0.585	0.317	0.005	0.161	0.329
	L × C	0.002	0.771	0.257	0.015	0.139	0.442

¹ Treatment means represent the average values of three tanks per treatment.

² Differences were regarded as significant when $P < 0.05$. L and C showed the main effect of each factor, and L × C indicated their interactive effect.