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

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Research Article

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Abbreviations:
CAT, catalase; MDA, malondialdehyde; SOD, superoxide dismutase

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Abstract

In this study, nine isonitrogenous experimental diets containing graded levels of carbohydrates (40 g/kg, 80 g/kg and 120 g/kg) and crude lipids (80 g/kg, 120 g/kg and 160 g/kg) were formulated in a two-factor (3 × 3) orthogonal design. A total of 945 mandarin fish with similar body weights were randomly assigned to twenty-seven 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 the survival rate and growth performance of mandarin fish. However, high dietary lipid and carbohydrate levels significantly decreased the protein content of the whole body and muscle of cultured fish. The lipid content of the 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 levels. 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 levels. Additionally, the increase in 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 carbohydrate levels for mandarin fish were 120 g/kg and 80 g/kg, respectively.

Mandarin fish (*Siniperca chuatsi*) is highly favoured among the 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 tonnes[(1)]. However, the mandarin fish largely rely on live bait, and in practice, about less than 10 % of the total production of compound feed has been applied in the cultivation of this fish species[(2)], which is 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 benefits to its cultivation sector.

Carbohydrates and lipids act as the main non-protein energy sources of aquafeed, and their potential protein-sparing effects have been well documented in teleosts[(3,4)]. Carbohydrates are the cheapest energy source and act 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 carbohydrates could lead to metabolic disorders, which further induce the reduction in growth and feed utilisation, 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 is also an important source 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 lipids and carbohydrates 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 carbohydrates and lipids in mandarin fish.

In recent years, with the improvement in living standards, the flesh quality of farmed fish has attracted more attention. In practice, some consumers believe that the flesh quality of farmed mandarin fish fed compound feed does 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 softens 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 the dietary carbohydrate and lipid levels and their interactions might also affect the flesh quality of mandarin fish.

Considering all of the above, a two-factor (3 × 3) orthogonal study was designed to investigate the effects of dietary carbohydrate and lipid levels on growth performance, hepatic histology and antioxidant capacity and flesh quality of mandarin fish. It is envisaged that the 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 levels of carbohydrates (40 g/kg, 80 g/kg and 120 g/kg) and crude lipids (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 gelatinisation (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 was 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 4 weeks' acclimation, a total of 945 fish with similar body weights (55 g) were randomly assigned to twenty-seven tanks (thirty-five fish/tank; water volume, 1000 litre). Triplicate tanks of fish were fed one of the experimental diets twice daily (07.00 and 16.30) for 10 weeks. During the feeding trial, the fish were gradually fed the diet with particle diameters 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, a siphon was used to remove the dirt from the bottom of the bucket and discharge the sewage from the filtration tank 2 h 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 were fasted for 24 h, and the fish for sampling were anaesthetised with eugenol (1:10 000). 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 the whole body, and the individual body length and weight of the remaining ten fish were measured for the calculation of condition factor. Then, the liver and visceral mass of six fish were separated for hepatosomatic index and viscerosomatic index 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 analysed based on the method in the Association of Official Analytical Chemists (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 the Kjeldahl nitrogen determination method was used to measure crude protein content (KD310, Opsis) (# 976.05; AOAC, 2003). Soxhlet extraction method was taken to measure crude lipid content (SX-360, Opsis) (# 920.29; AOAC, 2003). The potassium hydroxide/anthrone was used to measure hepatic glycogen content[22], following a commercial kit (Nanjing Jiancheng Bioengineering Institute).

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, malondialdehyde (MDA) content and the activity of superoxide dismutase (SOD) and catalase (CAT) were analysed using commercial kits following the instruction (Nanjing Jiancheng Bioengineering Institute; catalogue no.: total protein, A045–2–2; MDA, A003–1; total antioxidative capacity, A015–2–1; CAT, A007–1–1; SOD, A001–3). In detail, total antioxidant capacity was evaluated by measuring the content of Fe^{2+} reduced from Fe^{3+}, which could form a solid orange–red complex with phenanthroline analogues, and its antioxidant capacity could be measured by colorimetry[23]. The total SOD activity was measured with the ferricytochrome C method[24]. 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]. CAT activity was determined by

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

	80 crude lipid			120 crude lipid			160 crude lipid		
Carbohydrate level (g/kg)	40	80	120	40	80	120	40	80	120
White fish meal*	560·0	560·0	560·0	560·0	560·0	560·0	560·0	560·0	560·0
Shrimp meal*	30·0	30·0	30·0	30·0	30·0	30·0	30·0	30·0	30·0
Wheat gluten meal*	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0
Fermented soybean meal*	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0	40·0
Blood cell powder*	27·5	27·5	27·5	27·5	27·5	27·5	27·5	27·5	27·5
Squid paste*	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0
Beer yeast*	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0	20·0
Vitamin mixture†	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0
Mineral mixture‡	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0	12·0
$Ca(H_2PO_4)_2$*	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0
Phospholipid oil*	30·0	30·0	30·0	30·0	30·0	30·0	30·0	30·0	30·0
Soybean oil*	–	–	–	40·0	40·0	40·0	80·0	80·0	80·0
α-starch*	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*	80·0	60·0	40·0	80·0	60·0	40·0	80·0	60·0	40·0
Food attractant (Dimethyl-beta-propiothetin)	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5	0·5
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

*Supplied by Xinxin Tian'en Aquatic Feed Co. Ltd.
†Vitamin Premix (mg/kg diet): vitamin A (Retinol), 4.8; vitamin D_3, 0.2; vitamin K_3, 14·72; vitamin B_1, 17·80; vitamin B_2, 48; vitamin B_6, 29·52; vitamin B_{12}, 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.
‡Mineral Premix (mg/kg diet): Cu ($CuSO_4$), 2·00; Zn ($ZnSO_4$), 34·4; Mn ($MnSO_4$), 6·20; Fe ($FeSO_4$), 21·10; I ($Ca(IO_3)_2$), 1·63; Se (Na_2SeO_3), 0·18; Co ($CoCl_2$), 0·24; Mg ($MgSO_4 \cdot H_2O$), 52·7.

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 cm^3 and fixing with 4 % paraformaldehyde. Then the samples were dehydrated and embedded in paraffin wax. After that, the samples were sectioned at 5 μm thickness for sub sequent staining. The slides were deparaffinized and rehydrated with dimethyl benzene and graded concentrations of ethanol stained with haematoxylin and eosin in conjunction. Finally, the images were captured (40×) using the Olympus-DP73 optical microscope (Olympus) was used to capture images.

Flesh quality analysis

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

Statistical methods

All data were analysed using SPSS version 22. The interaction effects of dietary lipid and carbohydrate levels on cultured fish were analysed by factorial (two-way) ANOVA. If the interaction was not significant, the Tukey's multiple range test was followed to determine the effects of a 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 utilisation

Different dietary lipid and carbohydrate levels did not significantly affect the survival rate, final body weight, specific growth rate, feed intake, feed efficiency rate and protein efficiency rate of mandarin fish ($P > 0·05$) (Table 2). The fish fed a dietary lipid (160 g/kg) and carbohydrate level (120 g/kg) displayed significantly higher condition factor and viscerosomatic index compared with those

Table 2. Growth performance and feed utilisation 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 (%/d)	FER	PER	CF	VSI (%)	HSI (%)
Individual treatment means*											
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^{AB}	8·23^{B}	1·11^{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}

Table 2. (*Continued*)

Two-way ANOVA: *P*†									
L	0·798	0·324	0·579	0·284	0·121	0·069	0·369	< 0·001	0·822
C	0·780	0·675	0·712	0·613	0·264	0·259	0·161	0·003	0·004
L × C	0·869	0·985	0·637	0·832	0·345	0·488	0·450	0·328	0·182

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.
*Treatment means represent the average values of three tanks per treatment.
†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. Values (means, N = 3) with a different superscript letter are significantly different from the other dietary groups ($p < 0.05$).
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, g/cm^3) = 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.

fed a low dietary lipid level (80 g/kg) and a low carbohydrate level (40 g/kg), respectively, ($P < 0·05$) (Table 2). The hepatosomatic index was only affected by dietary carbohydrate level and significantly increased with increasing carbohydrate levels ($P < 0·05$) (Table 2).

Approximate body composition

The whole body and liver moisture content of mandarin fish decreased significantly with increasing dietary lipid levels ($P < 0·05$) (Table 3). No significant effects were observed on whole-body moisture content with increasing carbohydrate levels ($P > 0·05$) (Table 3). However, fish fed a carbohydrate level of 40 g/kg displayed significantly higher liver moisture content than those fed 80 g/kg and 120 g/kg carbohydrate ($P < 0·05$) (Table 3). High dietary lipid and carbohydrate levels significantly decreased the protein content of the whole body and muscle of cultured fish ($P < 0·05$) (Table 3); however, only the 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 the 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 interactions 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 levels ($P < 0·05$) (Table 4). Hepatic SOD activity increased significantly with increasing levels of lipid content in the feed ($P < 0·05$) (Table 4); however, it 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 levels were at 80 g/kg and 120 g/kg, and the dietary carbohydrate levels 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 (Fig. 1(a), (b), (d) and (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 (Fig. 1(c), (f) and (i)). At a high lipid level (160 g/kg), compared with a 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 (Fig. 1(g), (h) and (i)). At a high carbohydrate level

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

		Whole body				Liver				Muscle		
Lipid (g/kg)	Carbohydrate (g/kg)	Moisture	Protein	Lipid	Ash	Moisture	Protein	Lipid	Glycogen	Moisture	Protein	Lipid
Individual treatment means*												
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	$14·60^{ab}$	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		$74·86^{b}$	$16·83^{c}$	$3·27^{a}$	$4·33^{b}$	$74·01^{b}$	14·52	$3·90^{a}$	$5·33^{a}$	80·19	$18·14^{c}$	$0·85^{a}$
120		$74·00^{a}$	$16·30^{b}$	$5·07^{b}$	$4·48^{b}$	$73·53^{b}$	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*†												
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

*Treatment means represent the average values of three tanks per treatment.
†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. Values (means, N = 3) with a different superscript letter are significantly different from the other dietary groups ($p < 0.05$).

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 means*					
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*†					
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 antioxidative capacity; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase.
*Treatment means represent the average values of three tanks per treatment.
†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. Values (means, N = 3) with a different superscript letter are significantly different from the other dietary groups ($p < 0.05$).

(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 increased volume of lipid droplets, the disappearance of some cells nuclei and the necrosis of some hepatocyte tissues occurred (Fig. 1(f) and (i)).

Flesh texture quality

Muscle hardness of cultured fish decreased significantly with increasing levels 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 the 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 utilisation of cultured mandarin fish,

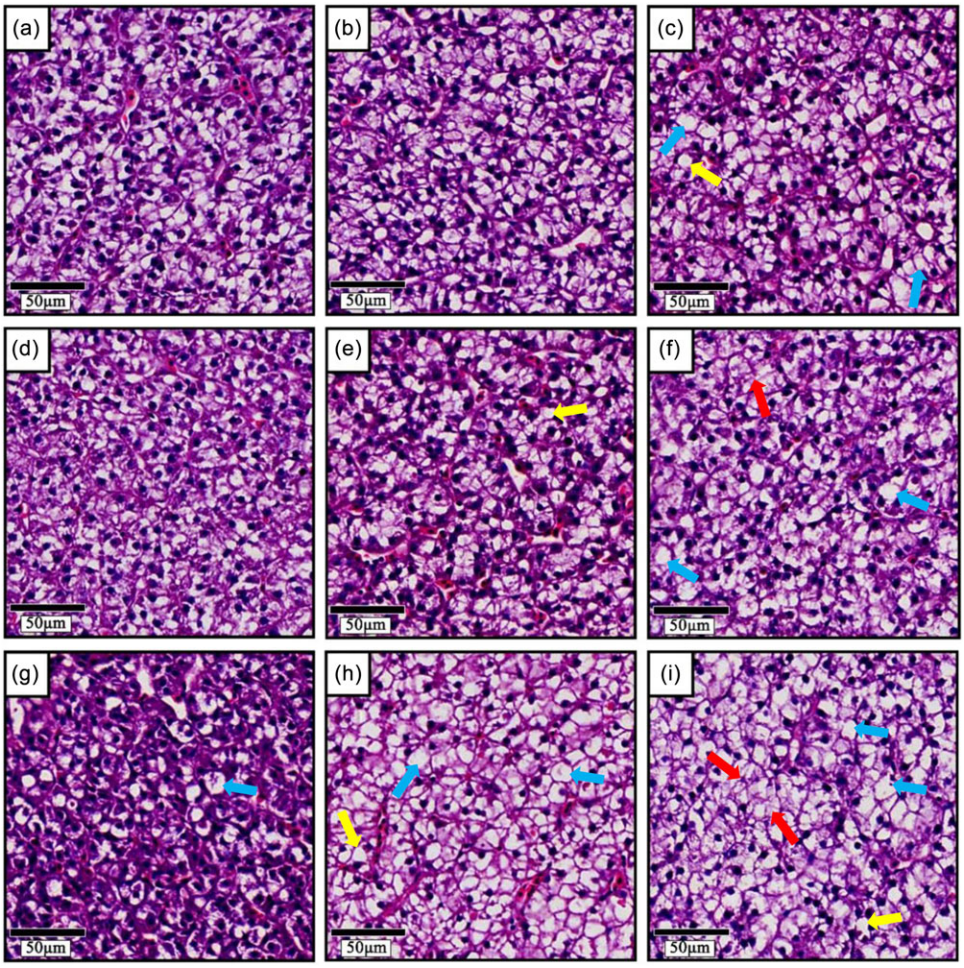

Figure 1. Effects of dietary lipid and carbohydrate interactions on the histomorphology of the liver of mandarin fish (40×). (a–c) 80 g/kg crude lipid. Carbohydrate levels were 40 g/kg, 80 g/kg, and 120 g/kg. (d–f) 120 g/kg crude lipid. Carbohydrate levels were 40 g/kg, 80 g/kg and 120 g/kg. (g–i) 160 g/kg crude lipid. Carbohydrate levels were 40 g/kg, 80 g/kg, and 120 g/kg. Lipid droplets (yellow arrow); swelling cells (green arrow); hepatocyte vacuolation (blue arrow); focal necrosis (red arrow).

including survival rate, specific growth rate, protein efficiency rate and feed efficiency rate, 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], large-scale shovel-jaw fish (*Onychostoma macrolepis*)[36] and loach (*Paramisgurnus dabryanus*)[37]. For carnivorous fish, limited glucose utilisation ability has 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 significantly affect the survival, growth performance and feed utilisation 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 levels exceeding 100 g/kg would negatively affect the growth performance of largemouth bass[41] and hybrid grouper[6], which might be because mandarin fish had a higher carbohydrate tolerance and the underlying mechanism required further exploration.

Although the growth performance and the feed utilisation 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 increasing trend with higher levels 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 in dietary carbohydrate level, and the same trend was found under each dietary lipid level, 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 levels at low carbohydrate levels; however, when carbohydrates reached 120 g/kg, liver glycogen was higher in the 120 g/kg and 160 g/kg lipid groups than in 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, liver injury could be observed in the high lipid and high carbohydrate groups. The increased dietary lipid and carbohydrate level also

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 means*							
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*†							
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

*Treatment means represent the average values of three tanks per treatment.
†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. Values (means, N = 3) with a different superscript letter are significantly different from the other dietary groups ($p < 0.05$).

reduced muscle protein content, and the increased dietary lipid level enhanced muscle lipid content, which indicated that dietary lipid and carbohydrate inclusion levels would also affect muscle compositions and further affect muscle growth of mandarin fish[47].

Increased liver lipid and glycogen content would affect the liver antioxidant capacity of aquatic animals[12,48]. 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 levels, which indicated the liver peroxidation damage induced by high dietary lipid and carbohydrate levels. 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 defence systems[50,51]. CAT could catalyse H_2O_2 decomposition, and SOD could catalyse superoxide radical dismutation, both of which could reflect reactive oxygen species 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 levels significantly affected muscle protein and lipid content, both of which were essential factors affecting flesh texture quality, the flesh texture quality indexes were further analysed[20,55]. In the present study, muscle hardness and resilience decreased with the increase of dietary lipid and carbohydrate levels, which might be related to the interaction of dietary lipid and carbohydrate, which induced a decrease in muscle protein content, and this result was in line with a 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 be 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 utilisation of mandarin fish, but high lipid and carbohydrate levels 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 levels 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.

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

References

1. Ministry of Agriculture and Rural Affairs, China (2024) *China Fishery Statistics Yearbook*. Beijing: China Agriculture Press.
2. Xiong Y & Lin L (2024) Domestication and breeding techniques of feed mandarin fish. *Sci Fish Farming* **08**, 76–77.
3. Kamalam BS, Medale F & Panserat S (2017) Utilisation of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations and future strategies. *Aquaculture* **467**, 3–27.
4. Turchini GM, Francis DS, Du ZY, *et al.* (2022) The lipids. In *Fish Nutrition*, pp. 303–467 [JE Halver and RW Hardy, editors]. London: Academic Press.
5. Gou SP, Chen NS, Xu XT, *et al.* (2015) Effects of dietary digestible starch levels on growth performance, body composition, and nonspecific immunological index of largemouth bass (*Micropterus salmoides*). *J Fish China* **39**, 1499–1510.
6. Li SL, Li ZQ, Zhang JC, *et al.* (2019) The impacts of dietary carbohydrate levels on growth performance, feed utilization, glycogen accumulation and hepatic glucose metabolism in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂). *Aquaculture* **512**, 734351.
7. Wang JT, Li XY, Han TY, *et al.* (2016) Effects of different dietary carbohydrate levels on growth, feed utilization and body composition of juvenile grouper *Epinephelus akaara*. *Aquaculture* **459**, 143–147.
8. Yan J, Liao K, Wang TJ, *et al.* (2015) 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* **10**, e0129937.
9. Xie SW, Lin YY, Wu TT, *et al.* (2021) Dietary lipid levels affected growth performance, lipid accumulation, inflammatory response and apoptosis of Japanese seabass (*Lateolabrax japonicus*). *Aquacult Nutr* **27**, 807–816.
10. Ma XH, Bi QZ, Kong YY, *et al.* (2022) Dietary lipid levels affected antioxidative status, inflammation response, apoptosis and microbial community in the intestine of juvenile turbot (*Scophthalmus maximus* L.). *Comp Biochem Phys A* **264**, 111118.
11. Castro C, Corraze G, Firmino-Diógenes A, *et al.* (2016) Regulation of glucose and lipid metabolism by dietary carbohydrate levels and lipid sources in gilthead sea bream juveniles. *Br J Nutr* **116**, 19–34.
12. Li SL, Li ZQ, Chen NS, *et al.* (2019) Dietary lipid and carbohydrate interactions: implications on growth performance, feed utilization and non-specific immunity in hybrid grouper (*Epinephelus fuscoguttatus*♀ × *E. lanceolatus*♂). *Aquaculture* **498**, 568–577.
13. Li SL, Yin J, Zhang HT, *et al.* (2019) Effects of dietary carbohydrate and lipid levels on growth performance, feed utilization, body composition and non-specific immunity of large yellow croaker (*Larimichthys crocea*). *Aquacult Nutr* **25**, 995–1005.
14. Lie Ø (2001) Flesh quality–the role of nutrition. *Aquac Res* **32**, 341–348.
15. Cheng JH, Sun DW, Han Z, *et al.* (2014) Texture and structure measurements and analyses for evaluation of fish and fillet freshness quality: a review. *Compr Rev Food Sci* **13**, 52–61.
16. Johnston IA, Li X, Vieira VLA, *et al.* (2006) Muscle and flesh quality traits in wild and farmed Atlantic salmon. *Aquaculture* **256**, 323–336.
17. Suárez MD, García-Gallego M, Trenzado CE, *et al.* (2014) Influence of dietary lipids and culture density on rainbow trout (*Oncorhynchus mykiss*) flesh composition and quality parameter. *Aquacult Eng* **63**, 16–24.
18. Wang Z, Qiao F, Zhang WB, *et al.* (2024) The flesh texture of teleost fish: Characteristics and interventional strategies. *Rev. Aquacult* **16**, 508–535.
19. Suárez MD, Martínez TF, Abellán E, *et al.* (2009) The effects of the diet on flesh quality of farmed dentex (*Dentex dentex*). *Aquaculture* **288**, 106–113.
20. Liu JH, Deng KY, Pan MZ, *et al.* (2020) Dietary carbohydrates influence muscle texture of olive flounder *Paralichthys olivaceus* through impacting mitochondria function and metabolism of glycogen and protein. *Sci Rep-UK* **10**, 21811.
21. AOAC (2003) *Official Methods of Analysis*, 17th ed. Washington, DC: Association of Analytical Communities.
22. Seifter S, Dayton S & Novic B (1950) The estimation of glycogen with the anthrone reagent. *Arch Biochem Biophys* **25**, 191–200.
23. Benzie IFF & Strain JJ (1999) Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Method Enzymol* **299**, 15–27.
24. Mccord JM & Fridovich I (1969) Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* **244**, 6049–6055.
25. Kei S (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* **90**, 37–43.
26. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.
27. Aebi H (1984) Catalase *in vitro*[M]//Methods in enzymology. *Academic Press* **105**, 121–126.
28. Xu XY, Yang H, Zhang CY, *et al.* (2021) Effects of replacing fishmeal with cottonseed protein concentrate on growth performance, flesh quality and gossypol deposition of largemouth bass (*Micropterus salmoides*). *Aquaculture* **548**, 737551.
29. Li L, Fang JG, Liang XF, *et al.* (2019) Effect of feeding stimulants on growth performance, feed intake and appetite regulation of mandarin fish, *Siniperca chuatsi*. *Aquac Res* **50**, 3684–3691.
30. Li L, Liang XF, Cai WJ, *et al.* (2021) Dietary with proper ratio of α-linolenic acid to linoleic acid enhanced the unsaturated fatty acids deposition of mandarin fish (*Siniperca Chuatsi*). *Aquacult Nutr* **27**, 73–85.
31. Wang XD, Li EC, Wang SF, *et al.* (2015) Protein-sparing effect of carbohydrate in the diet of white shrimp *Litopenaeus vannamei* at low salinity. *Aquacult Nutr* **21**, 904–912.

32. Tang J, Wang AL, Miao YT, *et al.* (2016) Protein-sparing effects and LPL gene expression of dietary lipids in the juvenile soft-shelled turtle, *Pelodiscus sinensis*. *Aquac Res* **47**, 579–590.
33. Francis SD & Turchini MG (2017) Retro-engineering the protein sparing effect to preserve *n*-3 LC-PUFA from catabolism and optimise fish oil utilisation: a preliminary case study on juvenile Atlantic salmon. *Aquaculture* **468**, 184–192.
34. Welengane E, Sado YR & Bicudo ADJÁ (2019) Protein-sparing effect by dietary lipid increase in juveniles of the hybrid fish tambatinga (♀ *Colossoma macropomum* × ♂ *Piaractus brachypomus*). *Aquacult Nutr* **25**, 1272–1280.
35. Jiang DY, Wang JT, Han T, *et al.* (2015) Effect of dietary lipid level on growth performance, feed utilization and body composition by juvenile red spotted grouper (*Epinephelus akaara*). *Aquacult Int* **23**, 99–110.
36. Gou NN, Chang ZG, Deng W, *et al.* (2019) Effects of dietary lipid levels on growth, fatty acid composition, antioxidant status and lipid metabolism in juvenile *Onychostoma macrolepis*. *Aquac Res* **50**, 3369–3381.
37. Wang ZR, Zhou SY, Zhang JH, *et al.* (2023) Effects of different protein and lipid levels on the growth performance and intestinal microflora of loach (*Paramisgurnus dabryanus*). *Anim Nutr* **13**, 229–239.
38. National Research Council (NRC) (2011) *Committee on the Nutrient Requirements of Fish and Shrimp, Nutrient Requirements of Fish and Shrimp*. Washington, DC: National Academies Press.
39. Qu HT, Ke W, Wen ZH, *et al.* (2022) Effects of dietary carbohydrate on growth, feed utilization, hepatic glucose and lipid metabolism in endangered Yangtze sturgeon (*Acipenser dabryanus*). *Aquac Res* **26**, 101334.
40. Xu TT, Liu XW, Huang WQ, *et al.* (2023) Effects of dietary carbohydrate levels on growth, metabolic enzyme activities and oxidative status of hybrid snakehead (*Channa maculata* ♀ × *Channa argus* ♂). *Aquaculture* **563**, 738960.
41. Liu YJ, Liu N, Wang A, *et al.* (2022) Resveratrol inclusion alleviated high-dietary-carbohydrate-induced glycogen deposition and immune response of largemouth bass, *Micropterus salmoides*. *Br J Nutr* **127**, 165–176.
42. Zhang WC, Dan ZJ, Zhuang YW, *et al.* (2022) Effects of dietary lipid levels on growth, digestive enzyme activities, antioxidant capacity, and lipid metabolism in turbot (*Scophthalmus maximus* L.) at three different stages. *Aquacult Nutr* **2022**, 1042263.
43. Berge GM, Zhou WW, Johansen LH, *et al.* (2023) Effects of dietary lipid level on growth, digestive physiology and disease resistance in lumpfish (*Cyclopterus lumpus*). *Aquaculture* **566**, 739209.
44. Chen YF, Cao KL, Huang HF, *et al.* (2023) Dietary effects of lipid and protein levels on growth, feed utilization, lipid metabolism, and antioxidant capacity of triploid rainbow trout (*Oncorhynchus mykiss*). *Aquacult Nutr* **2023**, 8325440.
45. Zheng LZ, Wang ZZ, Zhang B, *et al.* (2023) Effects of high dietary carbohydrate levels on growth performance, enzyme activities, expression of genes related to liver glucose metabolism, and the intestinal microbiota of *Lateolabrax maculatus* Juveniles. *Fishes-Basel* **8**, 431.
46. Xu HG, Dong XJ, Zuo RT, *et al.* (2016) Response of juvenile Japanese seabass (*Lateolabrax japonicus*) to different dietary fatty acid profiles: growth performance, tissue lipid accumulation, liver histology and flesh texture. *Aquaculture* **461**, 40–47.
47. Huang YY, Jiang GZ, Abasubong KP, *et al.* (2022) High lipid and high carbohydrate diets affect muscle growth of blunt snout bream (*Megalobrama amblycephala*) through different signaling pathways. *Aquaculture* **548**, 737495.
48. He YL, Tang YH, Xu N, *et al.* (2022) Effects of supplemental phytosterol on growth performance, body composition, serum biochemical indexes and lipid metabolism of juvenile large yellow croaker (*Larimichthys crocea*) fed with high lipid diet. *Aquaculture* **551**, 737899.
49. Ding XQ, Yuan CC, Huang YB, *et al.* (2021) Effects of phytosterol supplementation on growth performance, serum lipid, proinflammatory cytokines, intestinal morphology, and meat quality of white feather broilers. *Poult Sci* **100**, 101096.
50. Wang J, Zhang H, Zhang T, *et al.* (2015) Molecular mechanism on cadmium-induced activity changes of catalase and superoxide dismutase. *Int J Biol Macromol* **77**, 59–67.
51. Escobar JA, Rubio MA & Lissi EA (2015) Sod and catalase inactivation by singlet oxygen and peroxyl radicals. *Free Radical Bio Med* **20**, 285–290.
52. Hao ML & Liu RT (2019) Molecular mechanism of CAT and SOD activity change under MPA-CdTe quantum dots induced oxidative stress in the mouse primary hepatocytes. *Spectrochim Acta A* **220**, 117104.
53. Zhou YL, Guo JL, Tang RJ, *et al.* (2019) High dietary lipid level alters the growth, hepatic metabolism enzyme, and anti-oxidative capacity in juvenile largemouth bass *Micropterus salmoides*. *Fish Physiol Biochem* **46**, 125–134.
54. Xu HY, Han T, Li XY, *et al.* (2020) Effects of dietary lipid levels on survival, growth performance, and antioxidant ability of the early juvenile *Scylla paramamosain*. *Aquaculture* **528**, 735559.
55. Gong Y, Weng M, Wang XN, *et al.* (2023) Effects of vegetable oil replacement on intramuscular fat deposition and flesh quality of large yellow croaker (*Larimichthys crocea*) juveniles. *Aquaculture* **575**, 739731.
56. Zhou Y, Wu P, Jiang WD, *et al.* (2023) Dietary cinnamaldehyde improves muscle protein content by promoting muscle fiber growth via PTP1B/IGF1/PI3K/AKTs-TOR/FOXO3a signaling pathway in grass carp (*Ctenopharyngodon idella*). *Food Chem* **339**, 133799.
57. Jacob RH, & Pethick DW (2014) Animal factors affecting the meat quality of Australian lamb meat. *Meat Sci* **96**, 1120–1123.
58. Wang J, Su Y, Elzo MA, *et al.* (2016) Comparison of carcass and meat quality traits among three rabbit breeds. *Korean J Food Sci Anim Res* **36**, 84–89.