Physiological Metabolism and Antioxidant Capacity of Rainbow Trout (*Oncorhynchus Mykiss*) Fed Diets with Different Lipid Levels

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Research

Keywords: dietary lipid levels, rainbow trout, physiological metabolism, antioxidant capacity, recirculating aquaculture systems

Posted Date: September 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-78701/v1

Abstract

Background: In order to obtain the suitable lipid requirements of rainbow trout (*Oncorhynchus mykiss*) cultured in the recirculating aquaculture systems (RAS), the effects of dietary lipid levels on fat-related physiological metabolism and antioxidant defenses were investigated. Four isonitrogenous diets containing 45% crude protein with increasing dietary lipid levels of 12%, 15%, 18%, and 21% were fed to 360 fish with initial body weight of 333.25 ± 20.71 g for 77 d.

Results: The results showed that a high lipid diet increased lipase and amylase activities in trout livers, whereas a low lipid diet improved lipase and alkaline phosphatase activities in trout intestines. Meanwhile, increased dietary lipid levels elevated low density lipoprotein content and decreased high density lipoprotein, triglyceride, total cholesterol concentrations and the high density lipoprotein/low density lipoprotein ratio in serum. Moreover, increases in superoxide dismutase, glutathione, and malondialdehyde activities were observed in serum with increasing dietary lipid levels, while catalase activity and the catalase/superoxide dismutase ratio decreased.

Conclusion: These results indicate that rainbow trout fed diets with low and medium fat levels received adequate fat nutrition and that the diets ensured healthy growth by improving digestive absorption, fat synthesis and transport, and antioxidant levels, and the L12 group had the best growth performance. In conclusion, rainbow trout are healthier when fed diets with approximately 12% lipid levels in RAS.

Introduction

Given that fish face with more health problems due to the increase in stocking density and in environmental pressure, it has become more crucial to ensure fish health from the perspective of feed nutrition. Lipids are an important nutrient component of the fish diet, not only as a source of energy and essential fatty acids, but also as a carrier of fat-soluble vitamins. It also has a sparing action on dietary protein [1], as appropriate fat levels increase feed protein utilization. When dietary lipid levels are outside optimal levels, however, excessive body fat may accumulate, thereby affecting product quality and resulting in metabolic disorder [2–4]. Optimal dietary lipid levels promote digestive absorption [5], increase antioxidant abilities [6], and promote fat metabolism and immunity in plasma. These processes are highly beneficial in terms of nutrition metabolism and immunity for the generation of healthy aquaculture animals [7–9]. Therefore, it is important to supply optimal dietary lipid content to ensure healthy growth of fish.

Previous studies have investigated the lipid requirements of rainbow trout (*Oncorhynchus mykiss*) cultured in freshwater, but results are equivocal. Eliason et al. (2007) [10] reported that diets containing 15–20% lipid were appropriate for the optimal growth of rainbow trout, and Yigit et al. (2002) [11] reported that adult rainbow trout showed the best weight gain rates and feed conversion ratios with protein-caloric ratios of 22 mg/KJ, 44% protein level, and 17% lipid level. Eya et al. (2013) [12] reported that 42% protein and 20% lipid in the diet improved the growth of adult rainbow trout, with increased

activities of key enzymes in the hepatopancreas, intestine, and muscle. In another study, rainbow trout fed a diet containing 17.74% lipid grew better than those supplemented with 25.6% lipid [13]. These results show that optimum concentrations of lipids in the diet are closely related to dietary protein content, and the rainbow trout needs a relatively high lipid level to grow more healthily.

To date, there are no reports of the lipid requirements of rainbow trout cultured in the recirculating aquaculture systems (RAS). In this aquaculture setting, there are higher stocking density, and the lipid requirements for fish may need more accurate to decrease the harm of the oxidative damage and the stress response. Meanwhile, the strain experiences may excess the possibility of fat deposition to some extent [10], causing high abnormality rates, low growth rates, and high mortality, and whether these problems are due to excessive dietary lipid content remains to be determined.

In this study, in order to obtain the suitable lipid requirements of rainbow trout cultured in the RAS, the effects of dietary lipid levels on the digestive physiology, fat metabolism, and antioxidant capacity in the hepatopancreas and small intestine of fish were evaluated. The study may provide a theoretical basis and application guidance for the healthy culture of rainbow trout in the RAS.

Methods

Experimental animals and diets

360 adult rainbow trout (333.25 ± 20.71 g) were supplied by Oriental Ocean Sci-Tech Co., Ltd (Shandong Province, China). Fish were randomly distributed to 12 tanks (diameter: 95 cm, height: 100 cm and water depth: 65 cm) and acclimated for 21 d before the trail began, and there were 30 fish in each tank.

Four treatments with different dietary lipid levels (12%, 15%, 18%, and 21%, designated L12, L15, L18, and L21) with three replicates each were set. The crude protein of all diets was 45% based on dry matter. Fish oil was the only variable fat source. The trial feed (diameter: 5 mm) was processed by Beijing Hanye Science & Technology Co., Ltd (Beijing, China). Ingredients and proximate diet compositions are shown in Table 1.

Table 1
Main ingredients and chemical composition of trial diets

Ingredients (%)	Dietary lipid levels			
	L12	L15	L18	L21
Fish meal	36.00	36.00	36.00	36.00
Fish oil	4.76	7.86	10.95	14.09
Soybean meal	12.02	12.36	12.69	13.32
Corn gluten meal	8.26	8.78	9.30	10.00
Meat and born meal	10.00	10.00	10.00	10.00
Soy protein concentrate	6.00	6.00	6.00	6.00
Lecithin	4.00	4.00	4.00	4.00
Wheat flour	16.72	12.76	8.60	4.15
Sodium alginate	1.00	1.00	1.20	1.20
Multi-vitamin[a]	0.50	0.50	0.50	0.50
Multi-mineral[b]	0.50	0.50	0.50	0.50
Choline	0.25	0.25	0.25	0.25
Total	100	100	100	100
Nutrient levels[c]				
Dry matter	96.17	95.51	96.09	96.22
Crude protein	45.22	45.07	44.89	45.32
Ether extract	12.04	15.27	18.36	21.45
Protein lipid ratio	3.76	2.95	2.44	2.11
Calcium	2.31	2.29	2.31	2.30
Total phosphorus	1.63	1.63	1.62	1.61
Crude fiber	1.32	1.40	1.39	1.41

[a] Multi-vitamin (per kilogram diets): VA 7 500 IU, VC 100 mg, VD3 2 000 IU, VK3 5 mg, VB1 7.5 mg, VB2 15 mg, VB6 7.5 mg, VB12 0.02 mg, VE 50 mg, Nicotinic acid 6 mg, Calcium pantothenate 20 mg, Folic acid 1 mg, Biotin 0.12 mg, Inositol 500 mg.

[b] Multi-mineral (mg/kg diets): Cu 3; Zn 72; Mn 13; Fe 50; I 0.15; Co 0.5; Mg 600; Se 0.23; P 6000.

[c] Measured values except crude fiber.

Ingredients (%)	Dietary lipid levels			
	L12	L15	L18	L21
Ash	9.20	9.21	9.20	9.11
[a] Multi-vitamin (per kilogram diets): VA 7 500 IU, VC 100 mg, VD3 2 000 IU, VK3 5 mg, VB1 7.5 mg, VB2 15 mg, VB6 7.5 mg, VB12 0.02 mg, VE 50 mg, Nicotinic acid 6 mg, Calcium pantothenate 20 mg, Folic acid 1 mg, Biotin 0.12 mg, Inositol 500 mg.				
[b] Multi-mineral (mg/kg diets): Cu 3; Zn 72; Mn 13; Fe 50; I 0.15; Co 0.5; Mg 600; Se 0.23; P 6000.				
[c] Measured values except crude fiber.				

Growth trial

In the RAS, water circulated approximately 36 times and the new water input in each barrel accounted for about 10% of total water every day. The growth trial proceeded for 77 d. The average water quality was as follows: temperature 14 ± 0.5ºC; dissolved oxygen content 10 ± 0.5 mg L^{-1}; salinity 30 ± 0.5 g L^{-1}; pH 7.1 ± 0.2. Fish were fed to satiety four times per day and residual feed was collected and counted after 30 min of feeding.

Sampling

At the end of the trial, all fish after a 24 hour fast were performed with a rapid anesthesia by MS-222 (50 g/m^3) and weighed quickly. Six fish per tank were randomly chosen, then the blood was drawn from the caudal vein using 5 mL syringes and placed in a 10 mL centrifuge tube, which were rinsed in heparin sodium (0.5%). Blood samples were centrifuged at 5000 rpm for 15 min at 4 °C, after which they were kept still for 8 h at 4 °C to generate supernatants. The small intestine and hepatopancreas were quickly excised, frozen in ice, and stored at − 20 °C.

Analysis and measurement

Enzyme activities: Approximately 9 times PBS was added to tissue samples for thorough homogenization. Supernatants were set aside for activity measurements after centrifugation at 2500 rpm for 10 min. The following activities were measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu Province, China): protease, lipase (LPS), amylase (AMS), alkaline phosphatase (AKP), Na^+-K^+-ATPase, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malonaldehyde (MDA).

Plasma lipid metabolism and immune index: Activities of total cholesterol (TC) and triglyceride (TG) were also measured using commercial kits purchased from Nanjing Jiancheng Bioengineering Institute. High density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were measured using the direct method. Total protein (TP), globulin (GLO), and lysozyme (LZM) levels were assessed by using the biuret method, the biuret-bromocresol green method, and turbidimetry, respectively.

Calculations and statistical analysis

The following variables were calculated:

Weight gain rate (WGR, %) = 100 × (W_t - W_0) / W_0

Specific growth rate (SGR, % / day) = 100 × (lnW_t - lnW_0) / t

Daily feed intake (DFI, g/d) = feed consumed / number of fish / t

Feed conversion rate (FCR) = feed consumed / (W_t - W_0)

Where W_t and W_0 are final and initial fish weights, respectively; t is experimental duration in days; and feed consumed is calculated on a dry matter basis.

One-way analysis of variance was performed using SPSS 22.0 for Windows, followed by Duncan's multiple range tests. The mean values from each tank were used as the statistical unit in the analyses. Differences were regarded as significant at $P < 0.05$ and in some cases $P < 0.01$. All results are presented as mean ± standard deviation.

Results

Growth performance

Table 2 shows that that the L12 group had the best WGR, SGR and DFI, which were significantly higher than those of the L18 group by 34.35%, 29.31% and 14.61%, respectively ($P < 0.05$). Meanwhile, the L12 group also had the lowest FCR, which was significantly lower than those of the L18 and L21 group by 16.55% and 20.55%, respectively ($P < 0.05$). In conclusion, the L12 group had the best growth performance in this trail.

Table 2
Effects of dietary lipid levels on growth of rainbow trout

Groups	L12	L15	L18	L21
WGR (%)	77.41 ± 9.44^{a}	66.05 ± 6.21^{b}	57.62 ± 6.56^{b}	59.95 ± 7.57^{b}
SGR(%/d)	0.75 ± 0.05^{a}	0.67 ± 0.04^{ab}	0.58 ± 0.05^{b}	0.58 ± 0.06^{b}
DFI (g/d)	4.00 ± 0.23^{a}	3.35 ± 0.24^{b}	3.49 ± 0.22^{b}	3.68 ± 0.22^{ab}
FCR	1.16 ± 0.06^{b}	1.21 ± 0.11^{b}	1.39 ± 0.04^{a}	1.46 ± 0.09^{a}
Notes: WGR: Weight gain rate; SGR: Specific growth rate; DFI: Daily feed intake; FCR: Feed conversion rate				

Values are means ± SD. Means in the same line with different superscripts lowercase letters are significantly different ($P < 0.05$), and with alternate superscripts letters are highly significant difference ($P < 0.01$).

Digestive physiological indexes

Table 3 shows that protease, AMS, and LPS activities in the hepatopancreas increased as dietary fat level increased, but no significant differences in protease activity were detected among the groups ($P > 0.05$). AMS and LPS activities in the higher lipid groups (L18 and L21) were significantly higher than those the lower lipid groups (L12 and L15).

Table 3
Effects of dietary lipid level on digestive absorption of rainbow trout

Groups	L12	L15	L18	L21
Hepatopancreas				
Protease (U/mg prot)	101.20 ± 10.43^{a}	96.11 ± 2.43^{a}	109.16 ± 8.05^{a}	111.53 ± 6.22^{a}
AMS (U/mg prot)	0.10 ± 0.00^{b}	0.09 ± 0.01^{b}	0.13 ± 0.02^{a}	0.12 ± 0.00^{a}
LPS (U/mg prot)	37.03 ± 1.86^{c}	37.85 ± 2.35^{bc}	43.13 ± 2.13^{a}	41.93 ± 2.41^{ab}
Small intestine				
AMS (U/mg prot)	0.12 ± 0.01^{a}	0.13 ± 0.03^{a}	0.12 ± 0.01^{a}	0.14 ± 0.02^{a}
LPS (U/mg prot)	54.14 ± 1.32^{a}	51.09 ± 1.03^{b}	50.18 ± 1.03^{b}	50.25 ± 1.77^{b}
$Na^{+}K^{+}$-ATPase (U/mg prot)	0.57 ± 0.04^{a}	0.58 ± 0.06^{a}	0.54 ± 0.05^{a}	0.53 ± 0.01^{a}
AKP (kunit/g prot)	3.90 ± 0.07^{a}	3.82 ± 0.06^{a}	3.60 ± 0.08^{b}	3.51 ± 0.06^{b}
Notes: Protease: protease; AMS: amylase; LPS: lipase				

Values are means ± SD. Means in the same line with different superscripts lowercase letters are significantly different ($P < 0.05$), and with alternate superscripts letters are highly significant difference ($P < 0.01$).

LPS activity in the small intestine of the L12 group was significantly higher than that in the other three groups. There was no significant difference among groups in AMS activity in the small intestine ($P > 0.05$).

The effect of dietary fat levels on $Na^{+}K^{+}$-ATPase activity in the small intestine was not significant ($P > 0.05$). The activity of AKP decreased with increasing level of dietary fat.

Plasma lipid metabolism indexes

LDL-C levels increased in experimental fish as dietary fat level increased. For the L18 and L21 groups, LDL-C levels were significantly higher than levels in the L12 and L15 groups ($P < 0.05$). The L21 group showed significantly lower HDL-C level ($P < 0.05$) compared to the other groups, whereas no significant difference among the other three groups was detected for HDL-C levels ($P > 0.05$). The HDL-C/LDL-C ratio decreased with rising dietary fat level; however, the L21 group showed significantly lower HDL-C/LDL-C than the other groups ($P < 0.05$). TC content decreased with increasing dietary fat level; the L12 group showed a significantly higher level than the other three groups ($P < 0.05$) (Table 4). TG content increased initially, then decreased with increasing fat level; the L12 group had a significantly higher TG level than the other three groups ($P < 0.05$).

Table 4
Effects of dietary lipid level on fat metabolism indexes in plasma of rainbow trout

Groups	L12	L15	L18	L21
LDL-C (mmol/L)	4.15 ± 0.36^{b}	4.22 ± 0.35^{b}	4.83 ± 0.33^{ab}	5.03 ± 0.39^{a}
HDL-C (mmol/L)	0.94 ± 0.11^{a}	0.96 ± 0.05^{a}	1.00 ± 0.06^{a}	0.69 ± 0.07^{b}
HDL-C/LDL-C	0.23 ± 0.02^{a}	0.23 ± 0.02^{a}	0.21 ± 0.02^{a}	0.14 ± 0.01^{b}
TC (mmol/L)	8.81 ± 0.27^{a}	8.03 ± 0.10^{b}	7.96 ± 0.35^{b}	7.61 ± 0.39^{b}
TG (mmol/L)	4.86 ± 0.306^{a}	3.23 ± 0.105^{bc}	3.05 ± 0.120^{c}	3.55 ± 0.121^{b}
Notes: LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride				

Values are means ± SD. Means in the same line with different superscripts lowercase letters are significantly different ($P < 0.05$), and with alternate superscripts letters are highly significant difference ($P < 0.01$).

Antioxidant and immune indexes

Table 5 shows the antioxidant and immune indexes. SOD activity initially increased then stabilized with increasing level of dietary fat. The L15, L18, and L21 groups had significantly higher SOD levels than the L12 group ($P < 0.05$ or $P < 0.01$). CAT activity and the CAT/SOD ratio decreased with increased fat level. The L12 group showed significantly lower CAT levels than the L18 and L21 groups ($P < 0.05$ or $P < 0.01$), but no significant difference between the L12 and L15 groups was detected ($P > 0.05$). Group L12 had a significantly higher CAT/SOD ratio than the other three groups ($P < 0.05$). GSH content increased with increasing fat level, and the L21 group had a significantly higher level than the other three groups ($P > 0.05$).

Table 5
Effects of dietary lipid level on antioxidant and immune function of rainbow trout

Groups	L12	L15	L18	L21
SOD (U/mg prot)	403.22 ± 9.31^{c}	425.65 ± 6.33^{b}	446.11 ± 9.17^{a}	443.36 ± 10.20^{a}
CAT (U/mg prot)	24.74 ± 0.88^{a}	23.87 ± 0.41^{ab}	22.80 ± 0.62^{b}	20.72 ± 0.78^{c}
CAT/SOD	0.061 ± 0.00^{a}	0.056 ± 0.00^{b}	0.051 ± 0.00^{c}	0.047 ± 0.00^{d}
GSH (μmol/g prot)	6.09 ± 0.25^{b}	6.19 ± 0.44^{b}	6.68 ± 0.58^{b}	8.74 ± 0.21^{a}
MDA (nmol/mg prot)	0.51 ± 0.05^{b}	0.62 ± 0.08^{ab}	0.77 ± 0.07^{a}	0.71 ± 0.09^{a}
TP (g/L)	44.96 ± 1.23^{a}	44.62 ± 2.41^{a}	48.30 ± 3.01^{a}	47.73 ± 2.43^{a}
GLO (g/L)	22.45 ± 2.52^{a}	23.13 ± 1.24^{a}	23.88 ± 1.65^{a}	23.39 ± 0.52^{a}
LZM (U/ml)	1037.86 ± 79.2^{a}	1064.29 ± 57.14^{a}	945.71 ± 62.22^{a}	1009.52 ± 89.31^{a}
Notes: SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; MDA: malonaldehyde; TP: Total protein; GLO: globulin; LZM: lysozyme				

Values are means ± SD. Means in the same line with different superscripts lowercase letters are significantly different ($P < 0.05$), and with alternate superscripts letters are highly significant difference ($P < 0.01$).

Overall, MDA content showed an upward trend with increasing fat level in all groups except the L21 group, which showed a slight decrease. The L12 group had a significantly lower level than the L18 and L21 groups ($P < 0.05$), whereas there were no significant differences between the L12 and L15 groups ($P > 0.05$).

No significant differences in immune-related indexes (TP, GLO, and LZM) were detected among the treatment groups ($P > 0.05$).

Discussion

Effects of dietary fat levels on digestion and absorption

Fat is one of the main nutrients utilized by fish, and studying its effects on digestive and absorptive enzymes in the hepatopancreas and small intestine can help identify characteristics and features of the digestive physiology of fish. This research also provides a physiological basis for studying dietary fat demand characteristics of fish [5, 14, 15]. The effects of dietary fat levels on digestive enzyme activities in the hepatopancreas and small intestine of rainbow trout were different in this study. In the hepatopancreas, LPS and AMS activities increased initially and then stabilized with increased fat level (*P*

< 0.05). Overall, protease activity showed an upward trend with increased fat level in the diet ($P < 0.05$). In the small intestine, LPS activity decreased with increasing dietary fat level. The low fat group (L12) had a significantly higher LPS level than the other three groups ($P < 0.05$), and no significant differences were detected among those three groups ($P > 0.05$). These results suggest that increased dietary fat level was beneficial in improving the activity of LPS in the hepatopancreas. The fish may increase LPS secretion in response to increased dietary fat levels [5]; however, the fish may also balance the intake of carbon and nitrogen by promoting the secretion of proteases and AMS. On the other hand, increased dietary fat levels did not improve LPS activity at the small intestine. The L12 group displayed optimal LPS activity and showed that a low fat diet was beneficial in terms of LPS activity. It also showed that increasing fat levels were adverse for fat digestion.

In the small intestine, AKP activity was negatively correlated with increasing dietary fat level, as the L12 group had the strongest AKP activity. This observation suggests that low and mid levels of dietary fat could promote AKP secretion and promote the absorption of amino acids, lipids, glucose, and other nutrients [16]. However, there were no significant differences in Na^+K^+-ATPase activity among all groups ($P > 0.05$).

Overall, these results show that dietary fat levels affect digestive enzymes in different parts of the digestive tract in different ways and relatively independently of each other. Concerted increases in dietary fat levels appear to be beneficial for the increased secretion of digestive enzymes in the hepatopancreas, but they appear to be adverse for LPS activity in the small intestine. In the low fat groups (L12 and L15), the fish showed increased digestion and absorption of nutrients via enhanced LPS and AKP activity in the small intestine. It could be the digestive physiological characteristics or mechanism of the higher growth performance in the low fat group. These results show that low and medium dietary fat levels significantly improved the digestive physiology in this fish cohort.

Effects of dietary fat levels on fat metabolic indexes

The blood circulates nutrients around the body, and the content of HDL-C, LDL-C, TG, and TC can reflect the fat metabolism and nutritional status of fish [17]. Previous studies of the effects of dietary fat on blood lipid metabolism and immunity in rainbow trout focused on the fatty acid and lipid perspectives [18–20], and studies of dietary fat levels in these fish are rare [21].

In this study, LDL-C levels increased with increasing fat level, which indicates that cholesterol (CHO) output from the liver increased gradually. This pattern reflects a general increase in body tissue, as CHO content was found to have increased in peripheral tissue [22]. Similar findings were reported for juvenile hybrid sturgeon [23] and hybrid grouper [9]. High fat dietary supplementation also significantly decreased plasma HDL-C levels in the fish in the current study ($P < 0.05$). Thus, these data suggest that dietary fat levels significantly affected HDL-C synthesis in the liver and that a high fat diet was detrimental to CHO transport from peripheral tissue to the liver [22]. These data suggest a mechanism whereby lipid transport to the liver increases with increased dietary fat levels and that the liver transfers lipid components to other tissues through LDL-C synthesis to reduce lipid deposition.

The liver may also reduce CHO transport from peripheral tissues back to itself. The organ alleviates metabolic pressure, but it increases the burden on CHO metabolism in peripheral tissues. According to previous studies [22, 24], the HDL-C/LDL-C ratio reflects the transport direction of CHO. In this study, the HDL-C/LDL-C ratio of the high fat group (L21) was significantly lower than that of the other groups ($P < 0.05$). This observation suggests that CHO transport from the liver in L21 fish was high, thereby increasing CHO accumulation in peripheral tissues [25, 26]. However, the HDL-C/LDL-C ratio in the low and medium fat groups (L12, L15) was the highest among the treatment groups. A low ratio promotes the transport of CHO from peripheral tissues to the liver for metabolism, and it can lead to accumulation of CHO in the liver, which is beneficial to the health of fish.

TC and TG levels reflect the efficiency of fat metabolism in the liver (Om et al. 2003). The relationship between TC and TG content and dietary fat levels has been reported, but there are many differences among different fish species [21, 27]. In the current study, TC and TG contents were highest in the L12 group ($P < 0.05$), whereas the TG content in the L21 group was the second highest. These results suggest that fat anabolism in the livers of L12 fish was active and that its activity was beneficial in terms of dietary fat nutrition. Similar findings were reported for juvenile Nile tilapia [22]. A possible mechanism for this effect could be that the supply of exogenous CHO and TG from the L12 diet cannot meet bodily needs, therefore the fish strengthens endogenous synthesis of these lipids to meet growth demands. High fat diets can reduce TG synthesis in the liver [28] therefore the high TG content in group L21 very likely was exogenous.

In conclusion, dietary fat levels can influence CHO transport and the synthesis of lipids in the liver. When compared with the medium and high fat (L18, L21) diets, the low and medium fat (L12, L15) diets were advantageous for CHO transport to the liver, and provided more possibility for endogenous synthesis of TG and CHO. Low and medium fat diets may provide a smooth return of peripheral CHO metabolism to the liver by increasing HDL-C and decreasing LDL-C. The L12 diet could also increase CHO and endogenous synthesis or exogenous uptake of TG, providing adequate nutrition for the body. This characteristic may be one mechanism of fat metabolism for the two groups of fish that grew faster.

Effects of dietary fat levels on antioxidant and immunity

The liver undergoes fast metabolism and high oxygen consumption in vertebrates, which reflects the antioxidant defense capability of these organisms. The antioxidant system includes enzymatic systems and non-enzymatic systems. CAT and SOD belong to the former group, and GSH belongs to the latter group [29, 30].

In this study, SOD activity increased initially then stabilized with increasing level of dietary fat. SOD activity in the L18 group was the highest ($P < 0.05$ or $P < 0.01$). It has been suggested that concerted increases in dietary fat levels increase SOD activity, as similar findings were observed for juvenile turbot [15] and juvenile Chu's croaker [31]. Organisms can increase SOD activity with suitable increase of lipid. They convert $O_2^{\cdot -}$ to H_2O_2 and O_2. The H_2O_2 can be decomposed or utilized by CAT or peroxidase to avoid oxidative damage.

In this study, CAT activity decreased significantly with increased dietary fat level, which shows that the ability of CAT to scavenge H_2O_2 could be better at low fat levels. Similar findings were observed for the influence of dietary phospholipids on juvenile loach [6]. CAT may be more sensitive to dietary fat levels than SOD, such that a high fat diet inhibits CAT activity. However, Antonopoulou et al. (2014) [32] reported that CAT activity initially increased and then decreased with increasing fat level in the hepatopancreas of the Atlantic white croaker. This different result may be related to differences in animal species, test conditions, and experimental gradients. CAT activity in the L12 group in the current study was the highest of all groups, which suggests that CAT may have been efficient at removing excess H_2O_2 and scavenging O_2 indirectly in this group. Thus, a low fat diet may provide intrinsic conditions for the healthy growth of this cohort of fish. An increase in the CAT/SOD ratio suggests that antioxidant enzyme systems are activated to resist active oxygen species. On the contrary, the capacity of scavenging free radicals is reduced if the ratio was decreased [33]. This was consistent with our study results. CAT/SOD levels decreased significantly with increasing dietary fat level ($P < 0.05$). The L12 group had the strongest antioxidant capacity, indicating that a low dietary fat level improved the capacity for scavenging free radicals [34], which is consistent with growth.

GSH is the substrate for glutathione peroxidase and glutathione transferase. Organisms use these enzymes to scavenge free radicals and peroxides [35]. In this study, GSH synthesis in the liver increased with increased fat level ($P < 0.05$). Changes in GSH content were consistent with those of SOD, indicating that these two enzymes may cooperate in scavenging free radicals in fish. The change in GSH content was opposite to that of CAT, suggesting that antioxidant mechanisms in rainbow trout are variable.

MDA is a metabolite of fatty acid peroxidation; its presence is considered to be an indicator of liver damage [29]. In this study, MDA levels increased initially then decreased with concomitant increases in fat level. The L12 group had the lowest MDA level of all groups tested ($P < 0.05$). This result shows that lipid peroxidation in the L12 was the lowest, suggesting that the antioxidant capacity of this group was the strongest. This activity may relate to the action of CAT. Previous studies showed that MDA levels were positively correlated with dietary fat levels [15, 31, 36]. A possible mechanism for this effect is that intracellular fatty acids available for oxidation increase with increasing dietary fat level. The O_2 unused in this process may be converted to active oxygen radicals, thereby reacting with unsaturated fatty acids during membrane peroxidation. This process is harmful to membranes and produces large quantities of MDA.

Antioxidant pathways or mechanisms in test fish differed for different dietary fat levels. Low and medium fat groups (L12, L15) mainly depended on CAT to improve antioxidant abilities in the liver, thereby decreasing MDA levels. The medium and high fat groups (L18, L21) scavenged oxygen free radicals through the combined effects of SOD and GSH. Low and medium fat groups had the strongest antioxidant capacity through the coordination of CAT and SOD, thereby ensuring minimal oxidative damage to the cell membrane. This may be the mechanism that ensures the health of fish, and it may provide the internal conditions needed for higher growth performance.

LZM level in the blood is a common humoral immunity index in fish. GLO is an important molecule in the specific immune response, and TP content can reflect immunity status [7]. In this study, no significant differences in these immune-related indexes were detected among all groups ($P > 0.05$), suggesting that dietary fat levels had no significant effect on the blood immunity of fish in this study.

Conclusions

From the perspectives of digestion and absorption, lipid metabolism, and antioxidant activity, this study revealed potential metabolic characteristics of low and medium fat diets that promote healthy fish growth, and the L12 group had the best growth performance. Low and medium dietary fat levels can promote digestion and absorption of nutrients, anabolism and lipid transport, and increased antioxidant function in the liver of rainbow trout. These diets provide adequate fat nutrition for the body, ensuring healthy growth. In conclusion, under the RAS conditions with 45% dietary protein and 12% dietary fat, rainbow trout (330–600 g) grew well and were healthy.

Declarations

Acknowledgement

We thank the International Science Editing for its linguistic assistance during the preparation of this manuscript.

Authors' contributions

Y. Li designed research; J. Ma., Y.N. Lou., and J. Zhang. conducted research; J. Ma. and Y.N. Lou. analyzed data; J. Ma. wrote the paper. Y. Li and J. Ma. had responsibility for final content. The authors read and approved the final manuscript.

Availability of data and materials

The datasets produced and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Care of animals and experimental procedures were carried out following the approved CSIRO Animal Ethic Committee protocol No A12/2012.

Funding

This study was supported by the Twelfth Five-Year National Science and Technology Support Program of China (2011BAD13B07), and the Cooperation Projects of Chinese Academy of Sciences and Local Government (Y12530101L).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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