Effects of Dietary Protein and Lipid Levels on Growth Performance, Digestive Enzyme Activity, and Serum Biochemical Indices in Yadong Salmon Broodstock (Postprint)

Authors: Wang Changan, household country, Sun Peng, Gu Wei, Wang Bingqian, Xu Qiyou

Date: 2017-11-07T00:00:00+00:00

Abstract

This study aimed to investigate the effects of dietary protein and lipid levels on growth performance, gonadosomatic index (GSI), digestive enzyme activities, and serum biochemical parameters of Yadong salmon broodstock. A 5$×2two−factorcompletelyrandomizeddesignwasemployed,withfiveproteinlevels(36±$45.40) g were reared in indoor flow-through aquaria for a 77-day feeding trial. The results showed that at the same lipid level, weight gain rate increased with increasing protein levels, while feed conversion ratio decreased gradually, but increased again when protein level reached 48%. Dietary protein and lipid levels had no significant effect on female broodstock gonadosomatic index (P>0.05), while male broodstock gonadosomatic index decreased with increasing protein levels, but rebounded when protein level reached 48%. At the same lipid level, lipase activities in stomach, intestine, and pyloric ceca gradually increased with increasing dietary protein levels, being significantly higher at 48% protein level than at 36% protein level (P<0.05); whereas protease activities in stomach, intestine, and pyloric ceca showed an increasing trend, but with no significant differences among groups (P>0.05). At the same protein level, protease and lipase activities in stomach, intestine, and pyloric ceca of the 18% lipid group were slightly higher than those of the 9% lipid group. At the same lipid level, serum ammonia content gradually increased with increasing dietary protein levels. Dietary protein and lipid levels had no significant effects on serum superoxide dismutase, lysozyme activities, or glucose, triglyceride, total protein, albumin, and malondialdehyde contents (P>0.05). It was concluded that Yadong salmon broodstock exhibited certain adaptive responses to dietary protein (36%-48%) and lipid (9%-18%) levels; using weight gain rate as the indicator for broken-line model analysis, the dietary protein requirements for

Yadong salmon broodstock were determined to be 43.11% and 45.69% at lipid levels of 9% and 18%, respectively.

Full Text

Effects of Dietary Protein and Lipid Levels on Growth Performance, Digestive Enzyme Activities, and Serum Indices of Salmo trutta fario Broodstock

WANG Chang' an, HU Guo*, SUN Peng, GU Wei, WANG Bingqian, XU Qiyou

(Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Harbin 150070, China)

Abstract

This study investigated the effects of dietary protein and lipid levels on growth performance, gonadosomatic index (GSI), digestive enzyme activities, and serum indices of Salmo trutta fario broodstock. A 5$×2*two − factorrandomizeddesignwasemployed, withfiveproteinlevels*(36±$45.40) g were reared in indoor aquaria with flowing water for 77 days. The results showed that at the same lipid level, weight gain rate increased with increasing dietary protein level, while feed conversion ratio decreased gradually but increased again at the 48% protein level. Dietary protein and lipid levels had no significant effect on the GSI of female broodstock ($P>0.05$). The GSI of male broodstock decreased with increasing protein level but increased again at the 48% protein level. At the same lipid level, lipase activities in the stomach, intestine, and pyloric caeca increased gradually with increasing dietary protein level, with the 48% protein group being significantly higher than the 36% protein group ($P<0.05$). Protease activities in these tissues also increased, but no significant differences were observed among groups ($P>0.05$). At the same protein level, protease and lipase activities in the stomach, intestine, and pyloric caeca were slightly higher in the 18% lipid groups than in the 9% lipid groups. Serum ammonia content increased gradually with increasing dietary protein level at the same lipid level. Dietary protein and lipid levels had no significant effects on serum superoxide dismutase and lysozyme activities or on glucose, triglyceride, total protein, albumin, and malondialdehyde contents ($P>0.05$). In conclusion, Salmo trutta fario broodstock exhibited adaptive responses to dietary protein (36%-48%) and lipid (9%-18%) levels. Using weight gain rate as the indicator and broken-line model analysis, the dietary protein requirements were estimated to be 43.11% and 45.69% at lipid levels of 9% and 18%, respectively.

Keywords: Salmo trutta fario broodstock; protein; lipid; growth; digestive enzymes; serum biochemical indices

Introduction

Research on broodstock nutrition is of great practical significance for broodstock feed formulation and high-quality seed cultivation. Currently, knowledge of broodstock nutrition is limited, primarily due to the difficulty of research, which requires substantial aquaculture facilities, and feeding trials demand considerable time and financial investment [?]. Broodstock nutrition differs from that of early developmental stages, and the nutritional status of broodstock directly affects the nutritional condition of their offspring. While certain nutrients such as essential fatty acids have been identified as important for maintaining broodstock growth and reproductive performance, excessive or imbalanced levels can severely impact reproductive performance. However, information on the effects of most nutrients, such as trace elements and vitamins, on broodstock remains scarce.

Brown trout (*Salmo trutta*) is an important economic species among salmonid fish, prized for its delicious meat and high nutritional value. In China, it is only distributed in the Yadong region of Tibet, where it is known as Salmo trutta fario—a species considered synonymous with brown trout [?]. Foreign research on brown trout has been extensive, focusing primarily on biology and other aspects [?]. Currently, domestic research on the aquaculture biology of Salmo trutta fario is limited, concentrating mainly on molecular biology, reproductive biology, and culture methods [?]. No studies on its nutrition have been reported, and no specialized formulated feed exists. Feeding with feeds designed for other fish species often results in poor growth performance. Therefore, it is essential to develop efficient feeds for Salmo trutta fario to improve its growth performance and quality. Appropriate dietary protein and lipid levels are prerequisites for healthy fish growth and development and are two important parameters that must be prioritized in formulated feed design. This study investigated the effects of dietary protein and lipid levels on growth and reproductive performance, digestive enzyme activities, serum biochemical indices, and non-specific immunity of Salmo trutta fario broodstock to provide a theoretical basis for developing specialized broodstock feeds.

Materials and Methods

1.1 Materials Salmo trutta fario with an initial body weight of (462.53$±$45.40) g were provided by the Bohai Cold-Water Fish Experimental Station of Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Sciences.

1.2 Growth Trial A 5$×$2 two-factor completely randomized design was employed, with five protein levels (36%, 39%, 42%, 45%, and 48%) and two lipid levels (9% and 18%), resulting in ten practical experimental diets (P36/L9, P39/L9, P42/L9, P45/L9, P48/L9, P36/L18, P39/L18, P42/L18, P45/L18, P48/L18).

Each experimental diet was fed to three replicates of 19 fish. The composition and nutrient levels of the experimental diets are shown in Table 1 . Fish in each replicate were reared in indoor fiberglass tanks (diameter 90 cm, water depth 45 cm) with flowing water. Prior to the experiment, fish were acclimated to commercial feed for 14 days.

Before the experiment, fish were fasted for 24 h, anesthetized with phenoxyethanol (0.5 mL/L), and weighed and measured for body length. Experimental water was spring water with a temperature of 11.5–17.5 °C, pH 7.2–7.5, dissolved oxygen concentration of 7.8–10.0 mg/L, and ammonia concentration <0.2 mg/L. The photoperiod was 15 h light:9 h dark. Fish were fed twice daily at a feeding rate of 1.0% of body weight, with the principle of satiation without leftover feed. Residual feed was collected 40 min after each feeding. The feeding period lasted 77 days.

1.3 Sample Collection and Analysis

1.3.1 Growth Performance and Gonadosomatic Index At the end of the feeding period, fish were fasted for 24 h. The number of dead fish during the culture period and feed intake per tank were recorded, and the total weight of fish in each tank was measured. Six fish were randomly selected from each tank, dissected, and gonads were separated and weighed after sex identification. The entire viscera were also weighed. The following formulas were used to calculate growth and reproductive performance indices:

- Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0
- Survival rate (SR, %) = 100 × Nf / Ni
- Gonadosomatic index (GSI, %) = 100 × Wg / Wq

where W0 is initial body weight (g), Wt is final body weight (g), Nf is final number of fish, Ni is initial number of fish, Wg is gonad weight (g), and Wq is eviscerated body weight (g).

1.3.2 Digestive Enzyme Activities Four fish were randomly selected from each tank. The stomach, intestine, and pyloric caeca were sampled on an ice plate, washed with 0.86% pre-cooled physiological saline, blotted dry with filter paper, and weighed. The stomach, intestine, and pyloric caeca were homogenized (1:9 weight/volume) with 0.86% pre-cooled physiological saline using an FJ-200CL high-speed tissue homogenizer (15,000 r/min, 3 min), diluted, and centrifuged at 4,000 r/min for 10 min at 4 °C. The supernatant was collected in 1.5 mL centrifuge tubes and stored at -80 °C for digestive enzyme activity analysis. Protease activity was determined using the Folin-phenol method, amylase activity by the starch-iodine colorimetric method, lipase activity by the polyvinyl alcohol olive oil emulsion hydrolysis method, and tissue protein content by the Coomassie brilliant blue method [**?**]. Protease activity was defined as the amount of enzyme that hydrolyzed casein to produce 1 g of tyrosine per minute at 37 °C. Lipase activity was defined as the amount of enzyme that hydrolyzed fat to produce 1 g of fatty acid per minute at 37 °C. Amylase activity was defined as the amount of enzyme in 100 mL of enzyme solution that completely hydrolyzed 10 mg of starch within 30 min at 37 °C. Enzyme activities were expressed as units per gram of tissue protein (U/g prot).

1.3.3 Serum Indices Four fish were randomly selected from each tank and bled from the caudal peduncle. Blood was allowed to clot at 4 °C for 30 min and centrifuged at 3,000 r/min for 15 min. Serum was collected in 1.5 mL centrifuge tubes and stored at -80 °C. Serum total protein (TP), albumin (ALB), triglyceride (TG), and glucose (GLU) contents were measured using kits from Beckman Coulter Inc. with an automatic biochemical analyzer (Beckman ProCX4, USA). TP and ALB were determined by chemical methods, globulin (GLB) content was calculated as the difference between TP and ALB, and TG and GLU were determined by enzymatic methods. Serum ammonia (AMM), malondialdehyde (MDA) content, superoxide dismutase (SOD), and lysozyme (LZM) activities were measured using kits from Nanjing Jiancheng Bioengineering Institute. Enzyme activity units were defined as: the amount of enzyme that inhibited autoxidation of o-aminophenol by 50% per minute per mL of reaction solution at 37 °C for SOD; and the amount of enzyme that caused a decrease in absorbance of 0.001 per minute at 530 nm wavelength and pH 6.2 at 37 °C for LZM.

1.4 Statistical Analysis Results are expressed as mean ± standard deviation (mean±SD). Data were analyzed by two-way ANOVA using SPSS 19.0, followed by Duncan' s multiple comparison test with a significance level of $P<0.05$. Broken-line model analysis was performed using weight gain rate as the indicator.

Results

2.1 Effects of Dietary Protein and Lipid Levels on Growth Performance and Gonadosomatic Index As shown in Table 2 , dietary protein and lipid levels had no significant effect on survival rate of Salmo trutta fario broodstock ($P>0.05$). At 9% dietary lipid level, weight gain rate increased with increasing dietary protein level, while feed conversion ratio decreased initially but increased again at 48% protein level. At 18% dietary lipid level, weight gain rate increased initially with increasing protein level but decreased at 48% protein level, while feed conversion ratio decreased gradually with increasing protein level. At protein levels of 36%-42%, the 18% lipid groups showed higher weight gain rate and lower feed conversion ratio than the 9% lipid groups. At protein levels of 45%-48%, the 9% lipid groups showed higher weight gain rate and lower feed conversion ratio than the 18% lipid groups.

Dietary protein and lipid levels had no significant effect on the gonadosomatic index of female broodstock ($P>0.05$). At 9% lipid level, the gonadosomatic index of male broodstock generally decreased with increasing protein level, with a slight increase only at 42% protein level. At 18% lipid level, the gonadosomatic index of male broodstock decreased initially with increasing protein level but increased again at 48% protein level. At protein levels of 36%-42%, the 18% lipid groups showed higher male gonadosomatic index than the 9% lipid groups. At protein levels of 45%-48%, the 9% lipid groups showed higher male gonadosomatic index than the 18% lipid groups. Dietary protein and lipid levels had no significant interaction effects on weight gain rate, feed conversion ratio, or survival rate (P>0.05), but showed a significant interaction effect on male gonadosomatic index (P<0.05).

Broken-line model regression analysis based on weight gain rate indicated that at 9% dietary lipid level, the protein requirement was 43.11% (Y=2.97003X-75.683, $X\$ 43.11; Y = -0.5123X - 66.8357, X\ 43.11; R2 = 0.6862).At18\ 45.69; Y = -0.5269X + 71.8303, X\ \$45.69$; R2=0.7787).

2.2 Effects of Dietary Protein and Lipid Levels on Digestive Enzyme Activities As shown in Table 3 , at both 9% and 18% dietary lipid levels, lipase activities in the stomach, intestine, and pyloric caeca increased with increasing dietary protein level, with the P48/L18 group being significantly higher than the P36/L9 and P36/L18 groups (P<0.05). Protease activities in these tissues also increased, but no significant differences were observed among groups (P>0.05). At the same protein level, protease and lipase activities in the stomach, intestine, and pyloric caeca were slightly higher in the 18% lipid groups than in the 9% lipid groups. Dietary protein and lipid levels had no significant effect on amylase activity in the stomach, intestine, or pyloric caeca of Salmo trutta fario broodstock (P>0.05). No significant interaction effects were observed between dietary protein and lipid levels on digestive enzyme activities in different parts of the digestive tract (P>0.05).

2.3 Effects of Dietary Protein and Lipid Levels on Serum Biochemical Indices As shown in Table 4 , at 9% dietary lipid level, serum ammonia content increased gradually with increasing dietary protein level, reaching maximum values at 48% protein level, which was significantly higher than at 36% protein level (P<0.05). At 18% dietary lipid level, serum ammonia content also increased gradually with increasing protein level, with maximum values at 48% protein level, significantly higher than at 36% and 39% protein levels (P<0.05). At 9% lipid level, serum globulin content reached minimum values at 48% protein level, which was significantly lower than at 45% protein level (P<0.05), with no significant differences among other groups (P>0.05). At 18% lipid level, serum globulin content reached minimum values at 39% protein level, which was significantly lower than at 42% protein level (P<0.05), with no significant differences among other groups (P>0.05). Dietary protein and lipid levels had no significant effects on serum glucose, triglyceride, total protein, or albumin contents of Salmo trutta fario broodstock (P>0.05). No significant interaction effects were observed between dietary protein and lipid levels on serum ammonia, glucose, triglyceride, total protein, albumin, or globulin contents (P>0.05).

2.4 Effects of Dietary Protein and Lipid Levels on Serum Non-Specific Immune and Antioxidant Indices As shown in Table 5 , dietary protein and lipid levels had no significant effects on serum superoxide dismutase and lysozyme activities or malondialdehyde content (P>0.05). However, the low protein (36%) and low lipid (9%) group (P36/L9) showed the lowest serum superoxide dismutase and lysozyme activities and the highest malondialdehyde content. No significant interaction effects were observed between dietary protein and lipid levels on serum superoxide dismutase, lysozyme activities, or malondialdehyde content (P>0.05).

Discussion

3.1 Effects of Dietary Protein and Lipid Levels on Growth Performance and Gonadosomatic Index This study demonstrated that dietary protein level could be reduced from 48% to 42% without affecting growth performance or gonadosomatic index of Salmo trutta fario broodstock. Dietary protein is digested and decomposed into amino acids after ingestion, which then serve as substrates for protein synthesis or are partially used for energy consumption. The weight gain rate results indicated that the 36% protein group showed significantly lower growth performance than the 45%–48% protein groups, similar to results reported for grouper [?], primarily due to insufficient amino acid supply at low dietary protein levels. Weight gain rate increased significantly when dietary lipid level increased from 9% to 18%, indicating that 9% lipid level could not meet the growth requirements of Salmo trutta fario broodstock. Additionally, higher feed conversion ratios were observed at low protein levels, possibly because low-protein diets lack amino acids, particularly essential amino acids, leading to reduced feed utilization efficiency. Gonads must be closely linked to their corresponding ecological conditions for proper development, and inappropriate dietary protein and lipid levels can cause metabolic disorders in fish [?]. Under the conditions of this experiment, dietary protein and lipid levels had no significant effect on female gonadosomatic index, while male gonadosomatic index decreased with increasing protein level, similar to results reported for bighead carp [?] and green sea urchin [?]. Therefore, based solely on gonadosomatic index, 32% protein and 9% lipid levels might already meet the gonadal development requirements of Salmo trutta fario broodstock.

3.2 Effects of Dietary Protein and Lipid Levels on Digestive Enzyme Activities The effects of dietary composition on fish digestive enzyme activities are complex and diverse [?]. Studies have shown that within a certain range, dietary protein level is positively correlated with protease activity in fish such as yellow catfish [?], channel catfish [?], and spotted halibut [?]. In this study, protease activities in the stomach, intestine, and pyloric caeca of Salmo trutta fario broodstock increased with increasing dietary protein level at both 9% and 18% lipid levels. High dietary protein levels can induce the secretion of proteases in the fish digestive tract and enhance their activity, improving protein digestion capacity and promoting protein synthesis [?]. However, no significant differences were observed in protease activities among different parts of the digestive tract, similar to results for catfish [?], possibly because the relatively large size of Salmo trutta fario broodstock allowed endogenous protease secretion to meet the requirements of different protein levels (36%-48%). Within a certain range, digestive lipase activities in spotted halibut [?], yellow catfish [?], and Wuchang bream [?] decreased with increasing dietary protein level, while lipase activities in Indian major carp [?] and yellow catfish [?] were unaffected by dietary protein level, primarily related to consistent dietary lipid levels among experimental diets. In this study, lipase activities in the stomach, intestine, and pyloric caeca of Salmo trutta fario broodstock increased with increasing dietary protein level, indicating good utilization of both protein and lipid [?]. The effects of dietary protein level on amylase activity vary among fish species. Some fish show increased amylase activity with increasing dietary protein level, such as rainbow trout [?]. In this study, no significant changes were observed in amylase activities in different parts of the digestive tract of Salmo trutta fario broodstock, similar to results for gilthead sea bream [?], suggesting that amylase activity may not be directly related to dietary protein level but is primarily determined by genetic patterns and developmental processes.

The effects of dietary lipid level on lipase activity vary with species, season, and growth stage. Under the conditions of this experiment, lipase activities in the stomach, intestine, and pyloric caeca of Salmo trutta fario broodstock increased gradually with increasing dietary lipid level, consistent with results reported for brown rockfish [?], pejerrey [?], spotted rabbitfish [?], topmouth culter [?], and European sea bass [?], indicating that fish can enhance digestion and absorption of high-lipid diets by increasing lipase activity. Lipase is an inducible enzyme [?] affected by dietary lipid level [?], which may be the main reason for the positive correlation between lipase activities in different parts of the digestive tract and dietary lipid level in Salmo trutta fario broodstock. However, lipase activities in GIFT tilapia [?], flash catfish [?], and cobia [?] were unaffected by dietary lipid level, while lipase activity in red sea bream [?] decreased with increasing dietary lipid level. Additionally, this study showed that even at high dietary protein level (48%), lipase activities in different parts of the digestive tract were higher in the high-lipid (18%) groups than in the low-lipid (9%) groups, indicating that high dietary lipid level did not inhibit protein utilization in Salmo trutta fario broodstock (some studies suggest that excessive lipid can inhibit amino acid absorption and metabolism, reducing protein efficiency [?]). Dietary lipid level also affects protease and amylase activities. Low lipid levels can promote intestinal protease and amylase secretion in gibel carp [?], Chinese sucker [?], and discus fish [?], possibly because insufficient lipid supply prompts fish to enhance protein and carbohydrate digestion to maintain normal life activities.

For some fish species, protease and amylase activities in the digestive tract are highest within an appropriate lipid range and decrease when lipid levels are above or below the optimal range [?, ?]. Appropriate lipid levels maintain the strongest protease and amylase activities to enhance protein and carbohydrate digestion and absorption, while excessive lipid addition inhibits these enzyme activities. In this study, no significant differences were observed in protease and amylase activities in different parts of the digestive tract of Salmo trutta fario broodstock, possibly because protease and amylase activities were not affected by lipid level or were only minimally affected within the 9.0%–18.0% lipid range.

3.3 Effects of Dietary Protein and Lipid Levels on Serum Biochemical Indices In this study, dietary protein and lipid levels had no significant effect on serum glucose content, similar to results reported for GIFT tilapia [?] and meagre [?], indicating that carbohydrate metabolism in Salmo trutta fario broodstock was not affected by dietary protein and lipid levels. This may be because broodstock had sufficient glycogen reserves, preventing the reduction in blood glucose levels due to energy deficiency [?]. Additionally, dietary protein and lipid levels had no significant effect on serum total protein content, suggesting that broodstock may adapt to reproductive performance requirements by regulating protein homeostasis. Triglycerides are important components of blood lipids, and their content reflects hepatic lipid metabolism to some extent [?]. Serum triglyceride content tended to increase with increasing dietary lipid level in this study, similar to results for eel [?], Wuchang bream [?], and grass carp [?], indicating that dietary lipid not used for growth or reproduction is converted to lipid stores. When feed supply is insufficient, these lipid stores are mobilized to energy, promoting lipid synthesis in the reproductive system. When hepatic lipid accumulation reaches a certain level, blood triglyceride content decreases, as reported for Onychostoma sima [?] and GIFT tilapia [?], possibly because excessive dietary lipid causes nutritional fatty liver accumulation, damaging hepatocytes and reducing triglyceride synthesis capacity.

Serum ammonia content reflects protein catabolism in fish. Dietary protein is decomposed into amino acids, which enter the amino acid metabolic pool after absorption. Some amino acids are oxidatively decomposed to produce ammonia and urea, with ammonia being the main product of amino acid catabolism in fish. Accumulated ammonia is ultimately excreted through the gills [?]. Serum ammonia content in Salmo trutta fario broodstock increased with increasing dietary protein level, similar to results reported for large yellow croaker [?], Rhamdia quelen [?], and silver perch [?]. Under low protein conditions, serum ammonia content was significantly lower, indicating weaker protein catabolism primarily directed toward protein deposition and growth. When protein was excessive (48% protein level), protein synthesis decreased while catabolism increased, inhibiting protein deposition in Salmo trutta fario broodstock.

3.4 Effects of Dietary Protein and Lipid Levels on Serum Non-Specific Immune and Antioxidant Indices Dietary protein and lipid levels affect fish immune function. Studies have shown that serum lysozyme activity in rainbow trout increased significantly with increasing dietary protein level [?], while increasing dietary lipid level reduced respiratory burst activity of phagocytes in European sea bass [?] but enhanced serum lysozyme activity and leukocyte respiratory burst activity in grouper [?]. After challenge with *Aeromonas hydrophila* for 48 h, survival rates were higher in low and medium protein groups (23% and 28% protein) than in high protein groups (33% and 38% protein), and higher in low lipid groups (5% lipid) than in high lipid groups (8% and 11% lipid) in pale chub [?]. Therefore, appropriate dietary protein and lipid levels are important for maintaining fish immune function. However, different fish species show varying sensitivity to dietary protein and lipid levels. Hepatic damage occurred in grass carp when dietary lipid level exceeded 10% [?]. Salmo trutta fario is a carnivorous fish, and this study showed that serum lysozyme activity was not significantly higher at 18% lipid level than at 9% lipid level, suggesting that 18% lipid level may not have damaged non-specific immunity. Studies on common carp [?], gibel carp [?], and tilapia [?] showed that dietary protein level had no significant effect on serum lysozyme activity. Similar results were observed in this study, where serum lysozyme activity in Salmo trutta fario broodstock did not change significantly across protein levels of 33%–48%, possibly related to the growth stage. Previous studies focused primarily on larval and juvenile stages, while this experiment used broodstock that may have been less sensitive to the tested protein and lipid levels. Additionally, due to processing constraints, higher lipid levels were not tested in this study, and further research is needed to confirm the effects of high lipid levels on the immunity of Salmo trutta fario broodstock.

Superoxide dismutase and catalase primarily scavenge superoxide radicals, catalyzing the dismutation of hydrogen peroxide and reactive oxygen species. Reactive oxygen species can affect endogenous enzyme activities or membrane polyunsaturated fatty acids, leading to lipid peroxidation and malondialdehyde accumulation [?]. Dietary protein and lipid levels significantly affect fish antioxidant capacity. Within a certain range, decreasing dietary protein level increased serum and hepatopancreas superoxide dismutase and catalase activities in common carp, while malondialdehyde content increased significantly when protein level was below 26% [?]. Serum superoxide dismutase activity in gibel carp increased initially and then decreased with increasing dietary protein level [?], while it increased in koi carp [?] but decreased in gilthead sea bream [?]. Serum and liver superoxide dismutase activities in Atlantic salmon increased with increasing dietary lipid level, while serum malondialdehyde content decreased [?]. This study showed that dietary protein and lipid levels did not affect serum superoxide dismutase activity or malondialdehyde content in Salmo trutta fario broodstock, indicating no significant effect on antioxidant capacity. However, whether high lipid levels (>18%) would inhibit metabolism and accelerate peroxidation in Salmo trutta fario broodstock requires further investigation.

Conclusion

1. Within a certain range, appropriately increasing dietary protein and lipid levels can enhance lipase activity in the digestive tract of Salmo trutta fario broodstock, but protease and amylase activities do not change significantly.

2. When dietary protein and lipid levels range from 36%–48% and 9%–18%, respectively, Salmo trutta fario broodstock show significant changes only in serum ammonia and globulin contents, while other serum biochemical indices, non-specific immune indices, and antioxidant indices remain unchanged.

3. Using weight gain rate as the indicator, broken-line model analysis determined that the dietary protein requirements for Salmo trutta fario broodstock are 43.11% and 45.69% at lipid levels of 9% and 18%, respectively.

References

[1] IZQUIERDO M S, FERNÁNDEZ-PALACIOS H, TACON A G J. Effect of broodstock nutrition on reproductive performance of fish[J]. Aquaculture, 2001, 197(1/2/3/4): 25–42.

[2] CHANG Q, LIANG M Q, XUE H, et al. Research progress on broodstock nutrition[J]. Marine Fisheries Research, 2002, 23(2): 65–71.

[3] ZHANG C L, WANG W B. Preliminary study on fish in Tibet[J]. Acta Zoologica Sinica, 1962, 14(4): 529–536.

[4] VØLLESTAD L A, OLSEN E M, FORSETH T. Growth-rate variation in brown trout in small neighbouring streams: evidence density-dependence?[J]. Journal Biology, 2002, 61(6): 1513–1527.

[5] ASBJØRN VØLLESTAD L, MOLAND OLSEN E. Non-additive effects of density-dependent and density-independent factors on brown trout vital rates[J]. Oikos, 2008, 117(11): 1752–1760.

[6] PU D Y, WANG Z J, ZHAO H P, et al. Morphological and histological observation of the digestive system of Salmo trutta fario[J]. Sichuan Journal of Zoology, 2006, 25(4): 825–828.

[7] HAO F H, CHEN Y F, CAI B. Embryonic development of Salmo trutta fario in Tibet[J]. Journal of Fisheries of China, 2006, 30(3): 289–296.

[8] HAO F H, CHEN Y F, TANG W X, et al. Study on age and growth of Salmo trutta fario[J]. Acta Hydrobiologica Sinica, 2007, 31(5): 731–737.

[9] MENG W, YANG T Y, HAI S, et al. DNA barcoding of Salmo trutta fario based on mitochondrial COI gene sequences[J]. Chinese Journal of Fisheries, 2010, 23(1): 6–10.

[10] GUI Y M. Functional experiments of aquatic animals[M]. Beijing: China Agriculture Press, 2004: 92-98.

[11] JIANG S T, WU X Y, LI W F, et al. Effects of dietary protein and lipid levels on growth, feed utilization, body and plasma biochemical compositions of hybrid grouper (*Epinephelus lanceolatus* ×*Epinephelus fuscoguttatus*) juveniles[J]. Aquaculture, 2015, 446: 148-155.

[12] ZHANG Y L, SONG L, LIU R P, et al. Effects of dietary protein and lipid levels on growth, body composition and flesh quality of juvenile topmouth culter, *Culter alburnus* Basilewsky[J]. Aquaculture Research, 2016, 47(8): 2633-2641.

[13] SANTIAGO C B, CAMACHO A S, LARON M A. Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding floating cages[J]. Aquaculture, 1991, 96(2): 109-117.

[14] DAGGETT T L, PEARCE C M, TINGLEY M, et al. Effect of prepared and macroalgal diets and seed stock source on somatic growth of juvenile green sea urchins (*Strongylocentrotus droebachiensis*)[J]. Aquaculture, 2005, 244(1/2/3/4): 263-281.

[15] LI J S, LI J L, WU T T. Effects of dietary composition and environmental temperature on digestive enzyme activities of hybrid tilapia[J]. Journal of Fishery Sciences of China, 2004, 11(6): 585-588.

[16] SUN H C, XU J M, PANG M. Effects of dietary protein level on digestive enzyme activities of yellow catfish[J]. Journal of Hydroecology, 2010, 3(2): 84-88.

[17] JIANG G Z, LIU W B, WANG Y H, et al. Effects of dietary protein to lipid ratio on growth, digestive enzyme activities and muscle composition of juvenile channel catfish[J]. Journal of Fisheries of China, 2010, 34(7): 1129-1135.

[18] LÜ Y Y, CHEN S Q, YU C L, et al. Effects of dietary protein to lipid ratio on growth, digestive enzymes and serum biochemical indices of spotted halibut (*Verasper variegates*)[J]. Progress in Fishery Sciences, 2015, 36(2): 118-124.

[19] LIU Y, LI Y, GAO T T, et al. Effects of dietary lipid and protein nutrition on growth and muscle quality of Atlantic salmon in recirculating aquaculture systems[J]. Marine Sciences, 2013, 37(6): 47-59.

[20] LAZZARI R, RADÜNZ NETO J, PEDRON F D A, et al. Protein sources and digestive enzyme activities in jundiá (*Rhamdia quelen*)[J]. Scientia Agricola, 2010, 67(3): 259-266.

[21] HABTE-TSION H M, LIU B, GE X P, et al. Effects of dietary protein level on growth performance, muscle composition, blood composition, and digestive enzyme activity of Wuchang bream (*Megalobrama amblycephala*) fry[J]. The Israeli Journal Aquaculture, 2013, 65(3): 925-933.

[22] DEBNATH D, PAL A K, SAHU N P, et al. Digestive enzymes and metabolic profile of *Labeo rohita* fingerlings fed diets with different crude protein levels[J].

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2007, 146(1): 107-114.

[23] DE ALMEIDA L C, LUNDSTEDT L M, MORAES G. Digestive enzyme responses of tambaqui (*Colossoma macropomum*) fed on different levels of protein and lipid[J]. Aquaculture Nutrition, 2006, 12(6): 443-450.

[24] KAWAI S I, IKEDA S. Studies on digestive enzymes of fishes. 3. Development of the digestive enzymes of rainbow-trout after hatching and the effect of dietary change on the activities of digestive enzymes in the juvenile stage[J]. Bulletin of the Japanese Society of Scientific Fisheries, 1973, 39(7): 819-823.

[25] MOYANO F J, DÍAZ M, ALARCÓN F J, et al. Characterization of digestive enzyme activity during larval development of gilthead seabream (*Sparus aurata*)[J]. Fish Physiology and Biochemistry, 1996, 15(2): 121-130.

[26] YUE Y F, PENG S M, SHI Z H, et al. Effects of dietary lipid level on growth, intestinal digestive enzymes and major lipid metabolic enzyme activities of brown rockfish[J]. South China Fisheries Science, 2012, 8(6): 50-56.

[27] GÓMEZ-REQUENI P, BEDOLLA-CÁZARES F, MONTECCHIA C, et al. Effects of increasing the dietary lipid levels on the growth performance, body composition and digestive enzyme activities teleost pejerrey (*Odontesthes bonariensis*)[J]. Aquaculture, 2013, 416-417: 15-22.

[28] ZHANG L Z, ZHU W, WANG Y, et al. Effects of dietary lipid level on digestive enzyme activities and major blood biochemical indices of spotted rabbitfish[J]. Marine Fisheries, 2014, 36(2): 170-176.

[29] XIANG X, ZHOU X H, CHEN J, et al. Effects of dietary lipid level on digestive enzyme activities of juvenile topmouth culter[J]. Beijing Fisheries, 2008(5): 35-38.

[30] INFANTE J L Z, CAHU C L. High dietary lipid levels enhance digestive tract maturation improve *Dicentrarchus labrax* larval development[J]. The Journal Nutrition, 1999, 129(6): 1195-1300.

[31] ALIYU-PAIKO M, HASHIM R, SHU-CHIEN A C. Influence of dietary lipid/protein ratio on survival, growth, body indices and digestive lipase activity in Snakehead (*Channa striatus*, Bloch 1793) reared re-circulating water system[J]. Aquaculture Nutrition, 2010, 16(5): 466-474.

[32] MA X, HU Y, WANG X Q, et al. Effects of practical dietary protein to lipid levels on growth, digestive enzyme activities and body composition of juvenile rice field eel (*Monopterus albus*)[J]. Aquaculture International, 2014, 22(2): 749-760.

[33] HAN G M, WANG A M, XU P, et al. Effects of dietary lipid level on survival, muscle composition and digestive enzyme activities of juvenile GIFT tilapia[J]. Journal of Shanghai Ocean University, 2010, 19(4): 469-474.

[34] LEE H M, CHO K C, LEE J E, et al. Dietary protein requirement of juvenile giant croaker, *Nibea japonica* Temminck Schlegel[J]. Aquaculture Research, 2001, 32(Suppl.1): 112-118.

[35] TANG B G, CHEN G, ZHANG J D, et al. Effects of different feed series on growth, digestive enzyme activities and body composition of cobia larvae[J]. Acta Hydrobiologica Sinica, 2007, 31(4): 479-484.

[36] WANG Z G, CHEN P J, GU Y, et al. Effects of different feeds on digestive enzyme activities of red sea bream larvae[J]. Acta Oceanologica Sinica, 1998, 20(4): 103-106.

[37] MORAIS S, CONCEIÇÃO L E C, RØNNESTAD I, et al. Dietary neutral lipid level and source in marine larvae: effects digestive physiology intake[J]. Aquaculture, 2007, 268(1/2/3/4): 106-122.

[38] MOHANTA K N, MOHANTY S N, JENA J K, et al. Optimal dietary lipid level of silver barb, *Puntius gonionotus* fingerlings relation to growth, nutrient retention and digestibility, muscle nucleic acid content and digestive enzyme activity[J]. Aquaculture Nutrition, 2008, 14(4): 350-359.

[39] WANG A M. Study on the effects of dietary lipid level on growth and lipid metabolism regulation of GIFT tilapia[D]. PhD dissertation, Nanjing: Nanjing Agricultural University, 2011.

[40] WANG C M. Study on protein requirement of juvenile rock carp and lipid requirement of juvenile Chinese sucker[D]. Master' s thesis, Chongqing: Southwest University, 2011.

[41] WANG J Q, GENG J Z, JIANG Y S, et al. Effects of dietary lipid level on growth and digestion of juvenile discus fish[J]. Chinese Journal of Fisheries, 2009, 22(1): 24-30.

[42] FOUNTOULAKI E, ALEXIS M N, NENGAS I, et al. Effect of diet composition on nutrient digestibility and digestive enzyme levels of gilthead sea bream (*Sparus aurata* L.)[J]. Aquaculture Research, 2005, 36(13): 1243-1251.

[43] SHI G C. Effects of lipid on biochemical indices and fatty acid composition of GIFT tilapia at low temperature[D]. Master' s thesis, Zhanjiang: Guangdong Ocean University, 2012.

[44] CHATZIFOTIS S, PANAGIOTIDOU M, PAPAIOANNOU N, et al. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles[J]. Aquaculture, 2010, 307(1/2): 65-70.

[45] ZHOU M, WANG A L, XIAN J A. Variation of free amino acid and carbohydrate concentrations in white shrimp, *Litopenaeus vannamei*: effects of continuous cold stress[J]. Aquaculture, 2011, 317(1/2/3/4): 182-186.

[46] CHENG H L, XIA D Q, WU T T. Regulation of lipid metabolism and fatty liver in fish[J]. Chinese Journal of Animal Nutrition, 2006, 18(4): 294-298.

[47] LI X F, JIANG Y Y, LIU W B, et al. Protein-sparing effect of dietary lipid in practical diets for blunt snout bream (*Megalobrama amblycephala*) fingerlings: effects on digestive and metabolic responses[J]. Fish Physiology and Biochemistry, 2012, 38(2): 529-541.

[48] GAO P, JIANG M, WEN H, et al. Effects of different dietary protein to energy ratios on digestive enzyme activities of juvenile grass carp[J]. Freshwater Fisheries, 2009, 39(6): 54-58.

[49] XIANG X, ZHOU X H, CHEN J, et al. Effects of dietary lipid level on growth performance, body composition and serum biochemical indices of juvenile Onychostoma sima[J]. Chinese Journal of Animal Nutrition, 2013, 25(8): 1805-1816.

[50] LIN S Q, CHENG L, ZHANG W B, et al. Effects of dietary protein level on protein deposition and metabolic responses in large yellow croaker[J]. Journal of Ocean University of China, 2014, 44(5): 32-39.

[51] MELO J F B, LUNDSTEDT L M, METÓN I, et al. Effects of dietary levels of protein on nitrogenous metabolism of *Rhamdia quelen* (Teleostei: Pimelodidae)[J]. Comparative Biochemistry Physiology A: Molecular Integrative Physiology, 2006, 145(2): 181-187.

[52] YANG S D, LIOU C H, LIU F G. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*)[J]. Aquaculture, 2002, 213(1/2/3/4): 363-372.

[53] KIRON V, WATANABE T, FUKUDA H, et al. Protein nutrition and defence mechanisms in rainbow trout *Oncorhynchus mykiss*[J]. Comparative Biochemistry and Physiology Part A: Physiology, 1995, 111(3): 351-359.

[54] LIN Y H, SHIAU S Y. Dietary lipid requirement of grouper, *Epinephelus malabaricus*, and effects on immune responses[J]. Aquaculture, 2003, 225(1/2/3/4): 243-250.

[55] JIANG X H, LUO X N, JIN G H, et al. Effects of different dietary protein and lipid levels on growth, body composition and immunity of juvenile pale chub[J]. Chinese Journal of Animal Nutrition, 2016, 28(3): 864-871.

[56] JIN Y, TIAN L X, ZENG S L, et al. Dietary lipid requirement on non-specific immune responses juvenile grass carp (*Ctenopharyngodon idella*)[J]. Fish & Shellfish Immunology, 2013, 34(5): 1202-1208.

[57] LU X, CHENG Z Y, SUN J H, et al. Effects of reducing dietary protein level on growth, flesh quality and immunity of common carp[J]. Feed Industry, 2014, 35(6): 29-35.

[58] CAI C F, WU K, PAN X F, et al. Effects of protein nutrition on growth and immunity of gibel carp[J]. Acta Hydrobiologica Sinica, 2001, 25(6): 590-596.

[59] LE Y R, YANG H, XU Q Q, et al. Effects of dietary protein level on growth, immune function and disease resistance of hybrid tilapia (*Oreochromis niloticus*×*O. aureus*)[J]. Oceanologia et Limnologia Sinica, 2013, 44(2): 493-498.

[60] GÉRET F, JOUAN A, TURPIN V, et al. Influence of metal exposure on metallothionein synthesis and lipid peroxidation in two bivalve mollusks: the oyster (*Crassostrea gigas*) and the mussel (*Mytilus edulis*)[J]. Aquatic Living Resources, 2002, 15(1): 61-66.

[61] JIANG Z Q, SHI H Y, CUI P, et al. Effects of different protein levels of spirulina feed on color, growth and immunity of koi carp[J]. Journal of Northeast Agricultural University, 2012, 43(3): 95-103.

[62] MOURENTE G, DÍAZ-SALVAGO E, BELL J G, et al. Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata*) fed dietary oxidised oil: attenuation by dietary vitamin E[J]. Aquaculture, 2002, 214(1/2/3/4): 343-361.

Note: Figure translations are in progress. See original paper for figures.