Postprint on the Adaptability of Juvenile Epinephelus moara to High-Energy Low-Nitrogen Diets

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Abstract

To investigate the adaptability of juvenile clouded grouper (Epinephelus moara) to high-energy low-nitrogen diets, a two-factor experimental design was employed with protein levels of 35% (P35), 40% (P40), and 45% (P45), and lipid levels of 9% (L9), 12% (L12), and 15% (L15). Nine experimental diets were formulated and designated as P35L9, P35L12, P35L15, P40L9, P40L12, P40L15, P45L9, P45L12, and P45L15. A total of 810 juvenile clouded groupers with an initial average body weight of 27.09 g were randomly allocated into 9 groups, with 3 replicates per group and 30 fish per replicate. After 9 weeks of feeding, growth performance, muscle nutritional composition, and serum biochemical indices were determined. The results showed that: 1) With increasing dietary protein and lipid levels, the weight gain rate and specific growth rate of juvenile clouded groupers increased initially and then decreased, with a significant interaction between dietary protein and lipid levels ($P<0.05$). The P40L12 group exhibited the highest weight gain rate and specific growth rate at 184.59% and 1.49%/d, respectively. Feed conversion ratio, feeding rate, and protein efficiency ratio gradually decreased with increasing protein and lipid levels, with a significant interaction between dietary protein and lipid levels ($P<0.05$). No significant differences were observed in survival rates among all experimental groups ($P>0.05$). 2) Dietary lipid level had a significant effect on muscle crude lipid content ($P<0.05$), with the highest value observed in the L15 group. Dietary protein level, lipid level, and their interaction had no significant effects on muscle crude protein, moisture, and crude ash contents ($P>0.05$). 3) Serum aspartate aminotransferase activity increased with increasing dietary lipid level, with the highest activity in the L15 group (121.98 U/L), which was significantly different from the L9 group ($P<0.05$). Serum alanine aminotransferase activity increased with increasing dietary protein level, reaching its maximum value in the P45 group (89.79 U/L). Serum total cholesterol content showed a significant

positive correlation with dietary lipid level (P<0.05), but was not significantly affected by dietary protein level (P>0.05). Serum blood urea nitrogen showed a significant negative correlation with dietary protein level (P<0.05), with the P35 group exhibiting a value of 1.97 mmol/L, significantly higher than other protein level groups (P<0.05). Dietary protein level, lipid level, and their interaction had no significant effects on serum alkaline phosphatase activity and contents of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides (P>0.05). The results indicated that under the experimental conditions, a dietary protein level of 40% and lipid level of 12% yielded the optimal growth performance and relatively high feed utilization efficiency in juvenile clouded groupers.

Full Text

Adaptation of Juvenile Epinephelus moara to High-Energy, Low-Nitrogen Diets

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Abstract

A 9-week feeding trial was conducted to evaluate the adaptation of juvenile *Epinephelus moara* to high-energy, low-nitrogen diets. Nine practical diets were formulated using a 3$×$3 factorial design with three protein levels (35%, 40%, and 45%, designated P35, P40, and P45) and three lipid levels (9%, 12%, and 15%, designated L9, L12, and L15), resulting in nine treatment groups: P35L9, P35L12, P35L15, P40L9, P40L12, P40L15, P45L9, P45L12, and P45L15. Eight hundred ten healthy juvenile *E. moara* with an average initial body weight of 27.09 ± 2.07 g were randomly distributed into 27 experimental tanks (30 fish per tank), with each diet assigned to three replicate tanks. After the 9-week feeding period, growth performance, muscle nutritional composition, and serum biochemical parameters were measured.

The results showed: (1) Weight gain rate (WGR) and specific growth rate (SGR) initially increased then decreased with rising dietary protein and lipid

levels, with significant protein-lipid interactions observed for both parameters ($P < 0.05$). The P40L12 group achieved the highest WGR (184.59%) and SGR (1.49%/d). Feed conversion ratio (FCR), protein efficiency ratio (PER), and feed intake (FI) decreased progressively with increasing protein and lipid levels, also showing significant interactions ($P < 0.05$). Survival rates exceeded 98% across all groups and were not significantly affected by dietary treatments ($P > 0.05$). (2) Dietary lipid level significantly influenced muscle crude lipid content ($P < 0.05$), with the highest value observed in the L15 group. However, dietary protein and lipid levels and their interaction did not significantly affect muscle crude protein, moisture, or ash content ($P > 0.05$). (3) Serum glutamic-oxaloacetic transaminase (GOT) activity increased with dietary lipid level, reaching 121.98 U/L in the L15 group, significantly higher than the L9 group ($P < 0.05$). Serum glutamic-pyruvic transaminase (GPT) activity rose with increasing dietary protein level, peaking at 89.79 U/L in the P45 group. Serum total cholesterol (T-CHO) content correlated positively with dietary lipid level ($P < 0.05$) but was not significantly affected by protein level ($P > 0.05$). Serum urea nitrogen (UN) content showed a negative correlation with dietary protein level ($P < 0.05$), with the P35 group (1.97 mmol/L) significantly higher than other protein groups. Dietary protein and lipid levels and their interaction did not significantly affect serum alkaline phosphatase (AKP) activity or triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents ($P > 0.05$).

These results indicate that juvenile *E. moara* fed a diet containing 40% protein and 12% lipid exhibited optimal growth performance and feed utilization under the experimental conditions, with a protein-to-energy ratio of 20.37 mg/kJ and protein-to-lipid ratio of 3.13 mg/mg.

Keywords: juvenile *Epinephelus moara*; high-energy low-nitrogen diet; growth performance; muscle nutritional composition; serum biochemical parameters

1. Materials and Methods

1.1 Experimental Diets

A 3$×$3 factorial design was employed to formulate nine experimental diets using fish meal, soybean meal, and casein as protein sources, and fish oil and soybean oil as lipid sources. Three protein levels (35%, 40%, and 45%) and three lipid levels (9%, 12%, and 15%) were established, yielding diets designated P35L9, P35L12, P35L15, P40L9, P40L12, P40L15, P45L9, P45L12, and P45L15. Dietary formulations and proximate compositions are presented in Table 1 . Fish meal, soybean meal, fish oil, soybean oil, α-starch, and vitamin-mineral premixes were purchased from Qingdao Saigelin Biological Engineering Co., Ltd., while casein, carboxymethyl cellulose (CMC), choline chloride, and vitamin C phosphate were obtained from Qingdao Jinhaili Aquatic Technology Co., Ltd. Feed ingredients were ground to pass through a 60-mesh sieve, weighed

accurately according to formulation, and mixed thoroughly. The appropriate amounts of fish oil and soybean oil were added and mixed again, followed by addition of 35% water. The mixture was pelleted into 4 mm diameter particles, dried at 55°C, sealed in plastic bags, and stored at 0°C until use.

1.2 Experimental Design and Husbandry

Juvenile *E. moara* were obtained from Laizhou Mingbo Aquatic Co., Ltd., representing the same batch of offspring from artificial propagation. Eight hundred ten healthy juveniles with similar size (average initial weight 27.09 ± 2.07 g) were randomly allocated to 27 experimental concrete tanks (1.2 m^3 each) at a density of 30 fish per tank. Each diet was randomly assigned to three tanks (replicates). Fish were acclimated for one week prior to the formal experiment, which lasted nine weeks. Water temperature was maintained at 24 ± 1°C, salinity at approximately 30 ppt, with flow-through culture conditions. Fish were hand-fed to apparent satiation twice daily (09:00 and 16:00), with care taken to minimize feed waste. Daily feed intake, water temperature, pH, and salinity were recorded throughout the trial.

1.3 Sample Collection and Analysis

At the end of the experiment, fish were fasted for 24 h before final sampling. Fish in each tank were counted and weighed to calculate growth parameters. Five fish per tank were randomly selected, anesthetized with 100 mg/L MS-222, and approximately 2 mL of blood was collected from the caudal vein. Blood samples were stored at low temperature for 12 h, then centrifuged at 4,000 rpm for 10 min. The separated serum was stored at -20°C for subsequent biochemical analysis. An additional eight fish per tank were frozen for muscle proximate composition analysis.

Proximate composition of diets and muscle tissue was determined using standard methods: moisture by oven drying at 105°C, crude ash by muffle furnace incineration at 550°C, crude protein by Kjeldahl nitrogen analysis, and crude lipid by Soxhlet extraction with petroleum ether. Serum biochemical parameters were measured using commercial kits from Nanjing Jiancheng Bioengineering Institute. Glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and alkaline phosphatase (AKP) activities were determined by microplate methods. High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by direct dual-reagent methods. Triglyceride (TG) and total cholesterol (T-CHO) were assayed by single-reagent cholesterol oxidase-peroxidase (COD-PAP) methods. Urea nitrogen (UN) content was determined by diacetyl monoxime colorimetry.

1.4 Calculation Formulas

The following formulas were used to calculate growth and feed utilization parameters:

- Survival rate (SR, %) = 100 × (final fish number / initial fish number)
- Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight
- Specific growth rate (SGR, %/d) = 100 × (ln final body weight - ln initial body weight) / feeding days
- Feed conversion ratio (FCR) = total feed intake / (final body weight - initial body weight)
- Protein efficiency ratio (PER) = (final body weight - initial body weight) / total protein intake
- Feed intake (FI, %) = 100 × total feed weight / [(initial total weight + final total weight)/2 × feeding days]

1.5 Statistical Analysis

Experimental data were initially processed using Excel 2013, followed by two-way ANOVA using SPSS 17.0 to examine the main effects of protein level, lipid level, and their interaction on each parameter. When significant differences were detected, Duncan' s multiple range test was applied for post-hoc comparisons. Significance was set at $P < 0.05$. Data are presented as means ± standard deviation (SD).

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2. Results

2.1 Effects of High-Energy, Low-Nitrogen Diets on Growth Performance

Growth performance of juvenile *E. moara* is summarized in Table 2 . The P40 and P45 groups showed no significant differences in WGR, SGR, or PER ($P > 0.05$), but both were significantly higher than the P35 group ($P < 0.05$). FI and FCR decreased progressively with increasing dietary protein level, with FCR in P40 and P45 groups significantly lower than in P35 ($P < 0.05$). The L9 and L12 groups exhibited no significant differences in WGR, SGR, or FI ($P > 0.05$), but all were significantly higher than the L15 group ($P < 0.05$). PER in L12 and L15 groups did not differ significantly ($P > 0.05$) but were both significantly higher than L9 ($P < 0.05$). FCR decreased with increasing lipid level, with L15 significantly lower than L9 ($P < 0.05$). The P40L12 group achieved the highest WGR (184.59%), SGR (1.49%/d), and PER (2.25). Survival rates exceeded 98% across all treatments and were not significantly affected by dietary protein, lipid, or their interaction ($P > 0.05$). Significant protein-lipid interactions were observed for WGR, SGR, FI, FCR, and PER ($P < 0.05$).

2.2 Effects of High-Energy, Low-Nitrogen Diets on Muscle Nutritional Composition

Muscle proximate composition is presented in Table 3 . Muscle crude protein, moisture, and ash contents were not significantly affected by dietary protein, lipid, or their interaction ($P > 0.05$). Muscle crude lipid content initially decreased then increased with rising dietary lipid level, with L9 and L12 groups not differing significantly ($P > 0.05$) but both significantly lower than L15 ($P < 0.05$). Increasing dietary protein level did not significantly alter muscle crude lipid content ($P > 0.05$).

2.3 Effects of High-Energy, Low-Nitrogen Diets on Serum Biochemical Parameters

Serum biochemical parameters are shown in Table 4 . Dietary protein and lipid levels showed significant interactive effects only on serum GOT activity and UN content ($P < 0.05$). Serum GOT activity increased with both dietary protein and lipid levels, with lipid level exerting a significant main effect ($P < 0.05$). Serum GPT activity rose significantly with increasing protein level ($P < 0.05$). Serum AKP activity and HDL-C, LDL-C, and TG contents showed no significant differences across protein or lipid levels ($P > 0.05$). Serum T-CHO content increased with dietary lipid level, with L15 significantly higher than L9 and L12 ($P < 0.05$), but was not significantly affected by protein level ($P > 0.05$). The P35 group exhibited significantly higher serum UN content than P40 and P45 groups ($P < 0.05$), though no significant differences were observed among lipid levels ($P > 0.05$).

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3. Discussion

3.1 Effects on Growth Performance

Dietary protein level is a critical factor affecting fish growth performance. Within appropriate ranges, increasing dietary protein can significantly enhance growth rate, particularly in carnivorous fish species, though excessive protein is catabolized for energy, producing ammonia that pollutes the aquatic environment. In this study, WGR and SGR of juvenile *E. moara* increased with dietary protein level under low lipid conditions, consistent with findings in yellow catfish and starry flounder. As dietary protein and lipid levels increased, feed energy density rose, leading to gradual reductions in FI and FCR. The P40 group showed significantly higher WGR than P35, but did not differ from P45, with the P40L12 combination yielding the maximum WGR (184.59%), followed by P45L9 (181.92%). These optimal protein levels are lower than those reported for malabar grouper (47.8%) and hybrid grouper, likely reflecting species-specific differences or variations in diet formulation. The elevated lipid level in this study likely exerted a protein-sparing effect, resulting in an optimal

protein level of 40%, which aligns with Boonyaratpalin' s recommendation for practical grouper diets.

Lipids are essential nutrients for marine fish, providing energy, essential fatty acids, and facilitating absorption of fat-soluble vitamins. In this study, WGR, SGR, and PER in the L12 group exceeded those in L9, but further elevation to 15% lipid significantly reduced WGR and SGR while decreasing PER, consistent with results in juvenile rock carp, tiger puffer, and gibel carp. The optimal lipid level for juvenile *E. moara* appears to be 12%, which promoted growth and provided protein-sparing benefits, whereas excessive lipid reduced FI and inhibited rapid growth. This optimal level differs from some previous grouper studies, possibly due to variations in experimental design and diet composition.

3.2 Effects on Muscle Nutritional Composition

Excessive dietary lipid often leads to lipid deposition in liver, viscera, and muscle, impairing metabolism, compromising health, reducing flesh quality, and diminishing economic value. In this study, muscle crude lipid content in L15 was significantly higher than in L9 and L12, confirming this pattern. Muscle moisture and ash contents remained stable across treatments, consistent with studies on spring carp and turbot.

3.3 Effects on Serum Biochemical Parameters

GOT and GPT are key enzymes in amino acid metabolism and protein turnover. Under normal physiological conditions, serum transaminase activities are low, but liver damage increases cell membrane permeability, releasing these enzymes into circulation. Elevated serum GOT and GPT activities thus indicate hepatic dysfunction. In this study, high protein (P45) and high lipid (L12) groups showed elevated GPT activity, suggesting hepatic stress under these conditions. AKP regulates phosphorus and calcium metabolism, with its activity influenced by environment, nutrition, and physiological status. No significant differences in AKP activity were observed among treatments, suggesting minimal hepatic impairment.

UN represents the end product of protein metabolism and reflects protein utilization efficiency and amino acid balance. Lower serum UN indicates better amino acid balance and higher protein synthesis efficiency. The significantly higher UN content in P35 compared to P40 and P45 groups suggests reduced nitrogen retention and lower protein synthesis efficiency at suboptimal protein levels.

TG and T-CHO are primary blood lipids synthesized mainly in the liver, reflecting hepatic lipid metabolism. While some studies report elevated serum TG and T-CHO with high dietary lipid followed by declines at excessive levels due to hepatic steatosis, this study found serum T-CHO increased linearly with dietary lipid without significant changes in TG, suggesting maintained hepatic synthetic capacity across treatments. HDL-C and LDL-C mediate lipid

transport and metabolism, with HDL-C removing excess cholesterol from peripheral tissues and LDL-C delivering hepatic cholesterol to tissues. Although L15 showed slightly elevated HDL-C and LDL-C, differences were not significant, and no treatment induced marked dyslipidemia or cardiovascular risk indicators.

3.4 Conclusion

Comprehensive analysis of growth performance, muscle composition, and serum biochemical indicators indicates that juvenile *Epinephelus moara* achieve optimal growth when fed diets containing 40% protein and 12% lipid, corresponding to a protein-to-energy ratio of 20.37 mg/kJ and protein-to-lipid ratio of 3.13 mg/mg.

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