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Effects of Different Dietary Protein and Lipid Levels on Growth, Body Composition, and Immunity of Juvenile Phoxinus lagowskii: Postprint

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Abstract

To investigate the effects of dietary protein and lipid levels on growth, body composition, and immunity of juvenile Rhynchocypris lagowskii, twelve semi-purified diets were formulated using fish meal and soybean meal as protein sources and soybean oil as the lipid source, with protein levels of 23%, 28%, 33%, and 38% and lipid levels of 5%, 8%, and 11%, and fed to juvenile Rhynchocypris lagowskii with an initial average weight of (27.60$±$0.13) g. Each diet was fed to three net cages (replicates), with 30 experimental fish stocked per cage, for a 60-day culture trial. The results showed that dietary protein level had significant effects on weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) of juvenile Rhynchocypris lagowskii ($P<0.05$), dietary lipid level had significant effects on PER and FCR ($P<0.05$), but there was no significant interaction between dietary protein and lipid levels on any growth performance indicators ($P>0.05$). The WGR and SGR of the 23% and 28% protein groups were significantly higher than those of the 33% and 38% protein groups ($P<0.05$), the FCR of the 8% lipid group was significantly lower than that of the 11% lipid group ($P<0.05$), with no significant difference from the 5% lipid group ($P>0.05$). With increasing dietary protein level, the crude protein, crude lipid, and crude ash contents of whole body showed a trend of increasing first and then decreasing, reaching the maximum value at a protein level of 28%, while whole body moisture content was not affected by dietary protein level ($P>0.05$). With increasing dietary lipid level, whole body crude lipid content showed an increasing trend, but there was no significant difference between the 8% and 11% lipid groups ($P>0.05$). After 48 h of Aeromonas hydrophila challenge, the survival rate of the low and medium protein groups (23% and 28% protein groups) was higher than that of the high protein groups (33% and 38% protein groups), and the survival rate of the low lipid group (5% lipid group) was higher than that of the other two lipid

groups (8% and 11% groups). Based on the above results, the optimal protein to energy ratio for juvenile Rhynchocypris lagowskii diets is 23.67~26.03 mg/kJ, with a protein level of 23%~28% and a lipid level of 5%~8%.

Full Text

Abstract

To evaluate the effects of different dietary protein and lipid levels on growth, body composition, and immunity of juvenile Phoxinus lagowskii, twelve semi-purified diets were formulated with protein levels of 23%, 28%, 33%, and 38% (using fish meal and soybean meal as protein sources) and lipid levels of 5%, 8%, and 11% (using soybean oil as the lipid source). The feeding trial lasted 60 days, with each diet fed to three replicate net cages stocked with 30 juvenile fish each (initial average weight: (27.60$±$0.13) g). Results showed that dietary protein level significantly affected weight gain rate (WGR), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) ($P < 0.05$), while lipid level significantly affected PER and FCR ($P < 0.05$). No significant interactions between protein and lipid levels were observed for any growth performance indices ($P > 0.05$). Fish in the 23% and 28% protein groups exhibited significantly higher WGR and SGR compared to those in the 33% and 38% protein groups ($P < 0.05$). The 8% lipid group showed significantly lower FCR than the 11% lipid group ($P < 0.05$), though it did not differ significantly from the 5% lipid group ($P > 0.05$). Whole-body crude protein, crude lipid, and ash contents initially increased then decreased with rising dietary protein levels, peaking at 28% protein, while moisture content remained unaffected ($P > 0.05$). Whole-body crude lipid content increased with dietary lipid level, though no significant difference was observed between the 8% and 11% lipid groups ($P > 0.05$). Following challenge with Aeromonas hydrophila, survival rates at 48 h were higher in low- and medium-protein groups (23% and 28%) than in high-protein groups (33% and 38%), and higher in the low-lipid group (5%) than in the other two lipid groups (8% and 11%). Based on these findings, the optimal dietary protein-to-energy ratio for juvenile P. lagowskii is 23.67–26.03 mg/kJ, with recommended protein and lipid levels of 23%–28% and 5%–8%, respectively.

Keywords: juvenile Phoxinus lagowskii; protein; lipid; growth; body composition; immunity

Introduction

Phoxinus lagowskii, a small cyprinid fish belonging to the subfamily Leuciscinae, is commonly known as "liugen fish" and inhabits pristine mountain streams across major river systems in China, from the Heilongjiang River basin to the Yangtze River basin [**?**]. Valued for its tender flesh and nutritional quality, this species exhibits strong disease resistance and rapid growth, offering significant aquaculture potential. Previous research has addressed pond domestica-

tion, culture techniques, habitat suitability, and nutritional composition [**?**]. As an omnivorous species amenable to artificial feeding, P. lagowskii represents a promising candidate for commercial aquaculture. While nutritional requirements have been investigated for related species such as tench (Tinca tinca) [**?**], no studies have yet determined the optimal protein and lipid requirements for P. lagowskii. This study employs two-way ANOVA to systematically examine how varying dietary protein and lipid levels affect growth, body composition, and immune response, thereby establishing scientifically-based recommendations for feed formulation.

Materials and Methods

1.1 Experimental Ponds

The feeding trial was conducted at the Channel Catfish Breeding Farm of Qingnian Reservoir in Tieling County, Liaoning Province. The earthen pond covered approximately 6 mu (1 mu 667 m^2) with a water depth of 1.6 m. Thirty-six polyethylene net cages (370 cm × 170 cm × 140 cm, 30 mesh) were suspended from a wooden frame at the water surface, using high-density polyethylene foam as floats and stones as sinkers at the four corners.

1.2 Experimental Fish and Acclimation

A total of 1,080 healthy juvenile P. lagowskii with uniform body size (average weight: $(27.60\$\pm0.13)g; averagelength$: (11.60±$0.09) cm) were randomly distributed into 36 net cages at a stocking density of 30 fish per cage. The cages were randomly assigned to 12 dietary treatment groups, with three replicates per group. Fish were acclimated to the cages for one week and fed four times daily at 06:30, 10:30, 14:30, and 18:30.

1.3 Experimental Diets

Twelve experimental diets were formulated in a 4$×$3 factorial design with four protein levels (23%, 28%, 33%, 38%) and three lipid levels (5%, 8%, 11%). Fish meal and soybean meal served as protein sources, soybean oil as the lipid source, and dextrin as the carbohydrate source. All diets contained approximately 25% total carbohydrate, with protein-to-energy ratios ranging from 18.71 to 31.30 mg/kJ (microcrystalline cellulose was used as a filler and excluded from calculations). The diets were processed into 2.0 mm pellets using a small ring-die pellet mill. Dietary composition and nutrient levels are presented in .

1.4 Feeding Management

Water was sourced from Qingnian Reservoir. During the trial, water temperature was maintained at $(23\$\pm4)°CwithpHaround7.6.Eachcagewasequippedwithanairstoneprovidingcontinuohouraeration.Watertransparencyrangedfrom20–30cm, andammonianitrogen(NH\_\{3\}\$$-N) concentration remained below 0.4 mg/L. Fish were fed four times daily at

06:30, 10:30, 14:30, and 18:30. Feeding rates were adjusted based on residual feed and body weight changes, with fish weighed every 15 days. The daily ration was set at 5% of average body weight, and the trial duration was 60 days.

1.5 Challenge Test

At the end of the feeding trial, 25 fish from each group were selected for a challenge test with Aeromonas hydrophila, a pathogenic bacterium obtained from the Liaoning Key Laboratory for Prevention and Treatment of Aquatic Animal Diseases. The bacterium was activated twice and diluted with sterile physiological saline to a concentration of approximately 1$×10^{7}$ CFU/mL, as determined by preliminary tests. Fish were intraperitoneally injected with 1.0 mL bacterial suspension per 100 g body weight and transferred to plastic tanks with continuous aeration for mortality observation.

1.6 Sample Analysis

1.6.1 Growth Indices Growth performance was calculated using the following formulas: - Specific growth rate (SGR, %/d) = 100 × (ln W - ln W_0) / d - Weight gain rate (WGR, %) = 100 × (W - W_0) / W_0 - Protein efficiency ratio (PER, %) = (W - W_0) / (F × CP) - Survival rate (SR, %) = 100 × N / N_0 - Feed conversion ratio (FCR) = F / (W - W_0)

Where W is final body weight (g), W_0 is initial body weight (g), d is feeding duration (days), N is final fish number, N_0 is initial fish number, F is total feed intake (g), and CP is dietary crude protein content (%).

1.6.2 Proximate Composition Analysis Moisture, crude ash, crude protein, and crude lipid contents were determined by oven drying, muffle furnace incineration (550°C), Kjeldahl nitrogen analysis, and Soxhlet extraction, respectively. Total carbohydrate was measured by the 3,5-dinitrosalicylic acid method, and gross energy was determined using a microcomputer calorimeter [**?**].

1.7 Statistical Analysis

Data are expressed as mean ± standard error (mean±SE). Two-way ANOVA was performed using SPSS 17.0 software to analyze the effects of four protein levels and three lipid levels. One-way ANOVA followed by SNK and Duncan' s multiple comparison tests were used to identify differences among groups. Statistical significance was set at $P < 0.05$ [**?**].

Results

2.1 Effects of Dietary Protein and Lipid Levels on Growth Performance

Growth performance data after 60 days are summarized in . Dietary protein level significantly affected WGR, SGR, PER, and FCR ($P < 0.05$ or $P < 0.01$), while lipid level significantly affected PER and FCR ($P < 0.05$). No significant protein-lipid interactions were detected for any growth parameters ($P > 0.05$). The 28% protein group exhibited significantly higher WGR and PER than the other three protein groups ($P < 0.05$), along with significantly lower FCR ($P < 0.05$). Its SGR was also significantly higher than that of the 33% and 38% protein groups ($P < 0.05$). The 8% lipid group showed significantly higher PER than the other lipid groups ($P < 0.05$) and significantly lower FCR than the 11% lipid group ($P < 0.05$), though it did not differ significantly from the 5% lipid group ($P > 0.05$).

Based on overall growth performance, protein utilization, and feed efficiency, the optimal protein-to-energy ratio for juvenile P. lagowskii was determined to be 23.67–26.03 mg/kJ, with recommended dietary protein and lipid levels of 23%–28% and 5%–8%, respectively.

2.2 Effects of Dietary Protein and Lipid Levels on Body Composition

Whole-body proximate composition after 60 days is presented in . Dietary protein level significantly affected crude protein, crude lipid, and ash contents ($P < 0.05$), while lipid level significantly affected crude lipid and ash contents ($P < 0.05$). No significant protein-lipid interactions were observed for any body composition indices ($P > 0.05$). Whole-body crude protein, crude lipid, and ash contents initially increased then decreased with rising dietary protein levels, reaching maximum values at 28% protein. Whole-body crude lipid content increased with dietary lipid level, though no significant difference was found between the 8% and 11% lipid groups ($P > 0.05$).

2.3 Protective Effects Against Aeromonas hydrophila Infection

Twelve hours post-challenge, fish began exhibiting typical hemorrhagic septicemia symptoms including anal swelling and congestion at fin bases and body surfaces. Mortality peaked at 24 h, with 48-h survival rates of 76%, 64%, 60%, 84%, 60%, 52%, 48%, 36%, 40%, 44%, 36%, and 32% for groups 1–12, respectively. Statistical analysis revealed that fish fed diets containing 23%–28% protein and 5%–8% lipid showed higher survival rates, indicating enhanced disease resistance and immune function.

Discussion

3.1 Effects on Growth Performance

Protein and lipid are the most critical quantitative parameters in fish feed formulation. Dietary protein provides essential amino acids, while lipids supply essential fatty acids and facilitate absorption of fat-soluble vitamins [**?**]. Optimal protein and lipid levels are crucial for fish growth [**?**]. As an omnivorous cyprinid, P. lagowskii' s nutritional requirements have not been previously investigated. Excessive dietary protein wastes resources, pollutes water, and increases costs, while insufficient protein causes nutritional imbalances. Similarly, inappropriate lipid levels negatively affect growth.

Optimal protein-to-energy ratios vary widely among fish species due to differences in species, age, environmental conditions, feeding habits, protein source quality, non-protein energy source quality, and calculation methods for gross energy [**?**]. In this study, SGR and PER increased initially then decreased with rising protein levels, with maximum growth occurring at moderate rather than high protein levels, consistent with findings in snakehead (Channa striata) [**?**]. This suggests optimal protein-energy balance was achieved at moderate protein levels. Additionally, at optimal protein levels (28%), increased dietary lipid enhanced SGR and PER, demonstrating a protein-sparing effect of lipids, similar to results in Japanese seabass (Lateolabrax japonicus) [**?**]. Maximum SGR and PER along with minimum FCR were achieved at protein-to-energy ratios of 23.67–26.03 mg/kJ, protein levels of 23%–28%, and lipid levels of 5%–8%, which aligns with findings in crucian carp (Carassius auratus) [**?**] and suggests similar nutritional requirements among related cyprinid species. The absence of significant effects of protein and lipid levels on survival rate [**?**] corroborates our results.

3.2 Effects on Body Composition

Increased body protein content serves as a primary indicator of fish growth. In this study, whole-body crude protein content increased steadily from low (23%) to moderate (28%) protein levels, then decreased at high protein levels (33% and 38%). This suggests that maximum growth occurs only within an optimal protein-to-energy range; excess dietary protein is not utilized for tissue synthesis but catabolized for energy, consistent with studies on snakehead [**?**] and Chinese sturgeon (Acipenser sinensis) [**?**]. The data also revealed that at low-to-moderate protein levels (23% and 28%), whole-body crude lipid content increased with dietary lipid level, whereas at high protein levels (33% and 38%), this trend was not observed. This indicates that when protein requirements are not met or exceeded, non-protein energy sources (lipids, carbohydrates) cannot substitute for protein to enhance growth, supporting findings in European seabass (Dicentrarchus labrax) [**?**] and red drum (Sciaenops ocellatus) [**?**].

3.3 Effects on Immunity

Aeromonas hydrophila is a ubiquitous aquatic bacterium and primary pathogen for many aquatic animals, serving as a conditional pathogen for zoonotic disease. It proliferates at temperatures of 14.0–40.5°C and is commonly used in challenge tests to evaluate immunity, with survival rate serving as a comprehensive indicator [**?**]. In this study, typical hemorrhagic symptoms appeared 12 h post-injection, with mortality peaking at 24 h, consistent with previous research [**?**]. At 48 h, survival rates were higher in low- and medium-protein groups (23% and 28%) than in high-protein groups (33% and 38%), and higher in the low-lipid group (5%) than in the 8% and 11% lipid groups. Increased body lipid content correlates with reduced immunity [**?**], which aligns with our findings.

Conclusion

Based on comprehensive evaluation of growth performance, body composition, and immune response, the optimal dietary protein-to-energy ratio for juvenile Phoxinus lagowskii is 23.67–26.03 mg/kJ, with recommended protein and lipid levels of 23%–28% and 5%–8%, respectively.

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