Postprint of a Study on the Protein Requirement of Juvenile Hybrid Culter "Pioneer 1"

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Abstract

This experiment aimed to investigate the effects of dietary protein levels on growth performance, body composition, serum biochemical indices, and digestive enzyme activities of hybrid culter "Pioneer 1" juveniles, in order to determine their protein requirement. Six iso-lipidic and iso-energetic experimental diets with protein levels of 25.63%, 30.28%, 35.13%, 40.26%, 45.42%, and 50.53% were formulated using fish meal, casein, and gelatin as protein sources. A total of 630 hybrid culter "Pioneer 1" juveniles with an average body weight of (5.42±0.16) g were randomly divided into 6 groups with 3 replicates per group and 35 fish per replicate, and a 56-day culture trial was conducted in an indoor recirculating aquaculture system. The results showed: 1) With increasing dietary protein levels, the weight gain rate and specific growth rate of the experimental fish increased significantly first ($P<0.05$) and then stabilized after the dietary protein level reached 35.13%; feed conversion ratio showed a decreasing trend, with the lowest value in the 40.26% group, which was significantly lower than the first three groups ($P<0.05$); protein efficiency ratio and protein retention rate were both lowest in the 50.53% group, significantly lower than the first four groups ($P<0.05$). 2) Dietary protein level had no significant effect on moisture and crude ash contents of juvenile fish body ($P>0.05$), but significantly affected whole-body crude protein and crude lipid contents ($P<0.05$). Whole-body crude protein contents in the 40.26%, 45.42%, and 50.53% groups were all significantly higher than that in the 25.63% group ($P<0.05$); whole-body crude lipid content decreased significantly with increasing dietary protein levels ($P<0.05$), with the lowest value in the 50.53% group, significantly lower than the first five groups ($P<0.05$). 3) Dietary protein level had no significant effect on serum total cholesterol and triglyceride contents ($P>0.05$). Serum total protein content increased significantly first with increasing dietary protein levels ($P<0.05$), reached the highest in the 40.26% group, and then decreased; serum aspartate aminotransferase activity was highest in the 45.42% group, significantly higher than the

other four groups except the 40.26% group (P<0.05); serum alanine aminotransferase activity increased significantly first and then stabilized, with the 45.42% and 50.53% groups being significantly higher than the other groups (P<0.05). 4) With increasing dietary protein levels, protease activities in both intestine and liver increased significantly (P<0.05), while amylase activity showed a decreasing trend. Lipase activity showed no significant differences among groups (P>0.05). Using weight gain rate and protein efficiency ratio as evaluation indices, the dietary protein requirements of hybrid culter "Pioneer 1" juveniles were determined to be 36.43% and 38.81%, respectively, through broken-line regression analysis.

Full Text

Dietary Protein Requirement of Juvenile Erythroculter ilishaeformis ( ) × Ancherythroculter nigrocauda ( ) Hybrid F1

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Abstract: This experiment investigated the effects of dietary protein level on growth performance, whole body composition, serum biochemical indices, and digestive enzyme activities of juvenile Erythroculter ilishaeformis ( ) × Ancherythroculter nigrocauda ( ) hybrid F1 to determine their protein requirement. Six iso-lipidic and iso-energetic experimental diets were formulated using fish meal, casein, and gelatin as protein sources, with protein levels of 25.63%, 30.28%, 35.13%, 40.26%, 45.42%, and 50.53%. A total of 630 juvenile hybrid F1 with an average body weight of (5.42±0.16) g were randomly divided into 6 groups with 3 replicates each (35 fish per replicate) and cultured in an indoor recirculating aquaculture system for 56 days. The results showed: (1) Weight gain rate (WGR) and specific growth rate (SGR) increased significantly with increasing dietary protein level (P<0.05) and stabilized after reaching 35.13% protein. Feed conversion ratio (FCR) decreased, with the lowest value in the 40.26% group, which was significantly lower than the first three groups (P<0.05). Protein efficiency ratio (PER) and protein productive value (PPV) were lowest in the 50.53% group, significantly lower than the first four groups (P<0.05). (2) Dietary protein level had no significant effect on whole-body moisture or ash content (P>0.05), but significantly affected crude protein and crude lipid content (P<0.05). Whole-body crude protein in the 40.26%, 45.42%, and 50.53% groups was significantly higher than in the 25.63% group (P<0.05), while crude

lipid content decreased significantly with increasing protein level ($P<0.05$), with the 50.53% group showing the lowest value, significantly lower than the other five groups ($P<0.05$). (3) Serum triglyceride (TG) and total cholesterol (TCHO) contents were not significantly affected by dietary protein level ($P>0.05$). Serum total protein (TP) content increased significantly with increasing protein level ($P<0.05$), peaking in the 40.26% group before declining. Serum aspartate aminotransferase (AST) activity was highest in the 45.42% group, significantly higher than all groups except 40.26% ($P<0.05$). Serum alanine aminotransferase (ALT) activity increased initially and then stabilized, with the 45.42% and 50.53% groups significantly higher than the other groups ($P<0.05$). (4) Intestinal and hepatic protease activities increased significantly with increasing dietary protein level ($P<0.05$), while amylase activity decreased. Lipase activity was not significantly affected by dietary protein level ($P>0.05$). Broken-line regression analysis indicated that the dietary protein requirement of juvenile hybrid F1 was 36.43% and 38.81% based on WGR and PER, respectively.

Keywords: Erythroculter ilishaeformis ( ) × Ancherythroculter nigrocauda ( ) hybrid F1; protein; growth; requirement; serum biochemical index; digestive enzyme activity

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The hybrid F1 "Xianfeng No. 1" is a new culter fish variety developed through distant hybridization and molecular-assisted breeding technology, using selectively bred Erythroculter ilishaeformis as the female parent and selectively bred Ancherythroculter nigrocauda as the male parent. This variety was approved by the National Aquaculture Variety Approval Committee in 2012. It offers significant advantages including good body shape, fast growth rate, low culture cost, easy harvesting, and live fish transport, making it economically viable for pond aquaculture and suitable for nationwide promotion.

As a newly bred culter variety, the successful aquaculture of hybrid F1 "Xianfeng No. 1" requires high-quality formulated feed. However, research on its nutritional requirements remains scarce, necessitating systematic investigation. Protein is an essential nutrient for fish growth, development, and physiological activities, and represents the most costly component in aquafeeds. Insufficient dietary protein fails to meet growth requirements, while excessive protein is utilized for energy, causing resource waste and increased feed costs. Preliminary pond culture demonstrations by our team indicated that the protein requirement from large-size fingerling to adult stage is approximately 32%, but no studies have examined the juvenile stage. Previous reports show that juvenile Erythroculter ilishaeformis requires 41–43% or 48–54% dietary protein. Since hybrid F1 grows faster than both parental species, directly referencing these values is inappropriate. This study investigated the protein requirement of juvenile hybrid F1 using diets with varying protein levels to provide fundamental data for nutritional research and feed development.

1.1 Experimental Diets

Six iso-lipidic and iso-energetic semi-purified diets were formulated with protein levels of 25%, 30%, 35%, 40%, 45%, and 50% using fish meal, casein, and gelatin as primary protein sources, dextrin as the main carbohydrate source, and soybean oil and corn oil as lipid sources. The composition and nutrient levels are shown in Table 1 . All dry ingredients were ground and passed through a 60-mesh sieve, weighed according to the proportions in Table 1, and mixed thoroughly. Minor components were incorporated using the progressive enlargement method. Soybean oil and corn oil were added and mixed uniformly, followed by addition of appropriate water. The mixture was then pelleted (KL105A4-2, Chen' s Machinery Factory, Xinchang County, Zhejiang) into 2 mm diameter cylindrical pellets, air-dried indoors, crushed (SX-150, Shixin Machinery Factory, Shishou City, Hubei), and sieved to obtain particles of 0.5–1.2 mm. The final diets were packaged and stored at -20°C. The measured protein levels were 25.63%, 30.28%, 35.13%, 40.26%, 45.42%, and 50.53%.

1.2 Experimental Fish and Culture Management

Juvenile hybrid F1 were obtained from the National Hubei Wuhan Culter Breeding Farm. After transport to the indoor culture facility, fish were disinfected with 1% light saltwater and acclimated in concrete tanks for 2 weeks, during which they were fed a mixture of all experimental diets to adapt to the culture environment and feed. Prior to the experiment, fish were fasted for 24 h, and 630 healthy, uninjured fish of uniform size (average weight 5.42±0.16 g) were selected and randomly distributed into 6 groups with 3 replicates each (35 fish per replicate). The experiment was conducted in a recirculating aquaculture system consisting of 18 fiberglass tanks (400 L capacity, 82 cm diameter, 80 cm height, 75 cm water depth), sand filters, and water storage towers. The 56-day feeding trial involved feeding the six experimental diets three times daily (08:30–09:00, 12:30–13:00, 16:30–17:00) to apparent satiation. Sand filters were backwashed daily at 10:00, and feces were removed from tanks. Approximately one-quarter of the total system water volume was exchanged daily using aerated municipal tap water. Water temperature, feeding behavior, and mortality were recorded daily, with dead fish removed and weighed. During the trial, water temperature was 28–32°C, pH 7.3–7.6, dissolved oxygen >5 mg/L, NH -N <0.2 mg/L, under natural photoperiod.

1.3 Sample Collection

At the end of the feeding trial, fish were fasted for 24 h, counted, and weighed. Three fish were randomly selected from each tank, anesthetized in MS-222 solution (150 mg/L), measured for body length and weight, and blood was collected from the caudal vein into 1.5 mL centrifuge tubes. Blood samples were kept at 4°C for 2 h, centrifuged at 3,000 r/min for 10 min, and serum was collected. Fish were dissected on an ice tray; viscera and liver were weighed, and the midintestine was isolated. Liver and intestinal samples were stored at -80°C. An

additional three fish per tank were randomly selected for whole-body proximate analysis.

1.4 Parameter Measurements

Growth parameters were calculated as follows:
Weight gain rate (WGR, %) = 100 × (Mt - M0) / M0
Specific growth rate (SGR, %/d) = 100 × (ln Mt - ln M0) / T
Feed conversion ratio (FCR) = F / (Wt - W0)
Protein efficiency ratio (PER, %) = 100 × (Wt - W0) / (F × P)
Protein productive value (PPV, %) = 100 × (Wt × BPt - W0 × BP0) / (F × P)
Survival rate (SR, %) = 100 × Nt / N0
Viscerosomatic index (VSI, %) = 100 × Mv / Mw
Hepatosomatic index (HSI, %) = 100 × Mh / Mw
Condition factor (CF, %) = 100 × Mw / L^3

Where M0 and Mt are initial and final body weight (g); T is culture duration (d); F is feed consumption (g); W0 and Wt are initial and final total weight (g); P is dietary crude protein content (%); BP0 and BPt are initial and final whole-body crude protein content (%); N0 and Nt are initial and final fish number; Mv is viscera weight (g); Mh is liver weight (g); Mw and L are body weight (g) and length (cm).

Moisture, crude protein, crude lipid, and ash contents in feed and whole-body samples were determined by oven drying at 105°C, Kjeldahl method, Soxhlet extraction, and muffle furnace incineration, respectively. Serum triglyceride (TG), total cholesterol (TCHO), total protein (TP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a Sysmex Chemix-800 automatic biochemical analyzer.

Intestinal and liver samples were thawed at 4°C, and 1 g of tissue was homogenized with 0.65% saline at a 1:9 weight/volume ratio on ice, centrifuged at 3,000 r/min for 10 min, and the supernatant was used for enzyme activity and protein concentration assays. Protease activity was determined by the Folin-phenol method, amylase by iodine-starch colorimetry, lipase by microplate assay, and protein concentration by Coomassie brilliant blue method using kits from Nanjing Jiancheng Bioengineering Institute.

1.5 Data Analysis

Data were analyzed by one-way ANOVA using SPSS 18.0 software. Duncan' s multiple range test was used to determine significant differences among groups ($P<0.05$). Results are presented as means ± standard deviation. Broken-line regression analysis was used to estimate the protein requirement of juvenile hybrid F1.

2.1 Effects of Dietary Protein Level on Growth Performance and Feed Utilization

As shown in Table 2 , WGR and SGR increased significantly with increasing dietary protein level ($P<0.05$) and plateaued after reaching 35.13% protein, with no significant differences among the 35.13–50.53% groups ($P>0.05$). FCR showed the opposite trend, decreasing significantly ($P<0.05$) and stabilizing after 40.26% protein, with no significant differences among the 40.26%, 45.42%, and 50.53% groups ($P>0.05$). PER and PPV showed no significant differences among the first four groups ($P>0.05$), but decreased from the 45.42% group onward, with the lowest values in the 50.53% group, significantly lower than the first four groups ($P<0.05$).

VSI and HSI decreased with increasing dietary protein level, with the 45.42% and 50.53% groups showing significantly lower VSI than the first four groups ($P<0.05$). The 50.53% group had the lowest HSI, significantly lower than the 25.63%, 30.28%, and 40.26% groups ($P<0.05$). Dietary protein level had no significant effect on condition factor or survival rate ($P>0.05$). Broken-line regression analysis using dietary protein level as the independent variable and WGR or PER as dependent variables indicated that the dietary protein requirement of juvenile hybrid F1 was 36.43% (Figure 1 [Figure 1: see original paper]) and 38.81% (Figure 2 [Figure 2: see original paper]), respectively.

2.2 Effects of Dietary Protein Level on Whole-Body Composition

As shown in Table 3 , dietary protein level had no significant effect on whole-body moisture or ash content ($P>0.05$). Whole-body crude protein tended to increase with dietary protein level, with the 40.26%, 45.42%, and 50.53% groups significantly higher than the 25.63% group ($P<0.05$), but no significant differences among these three groups ($P>0.05$). Whole-body crude lipid decreased significantly, with the 40.26% and 45.42% groups significantly lower than the first three groups ($P<0.05$), and the 50.53% group significantly lower than all other groups ($P<0.05$).

2.3 Effects of Dietary Protein Level on Serum Biochemical Indices

As shown in Table 4 , dietary protein level had no significant effect on serum TG or TCHO content ($P>0.05$), but significantly affected TP content and AST and ALT activities ($P<0.05$). Serum TP content increased initially and then decreased, peaking in the 40.26% group, which was significantly higher than all other groups ($P<0.05$). Serum AST activity increased significantly from 25.63% to 45.42% protein ($P<0.05$), then decreased at 50.53% protein, becoming significantly lower than the 45.42% group ($P<0.05$). Serum ALT activity increased and plateaued at 45.42% protein, with the 45.42% and 50.53% groups significantly higher than other groups ($P<0.05$), but not significantly different from each other ($P>0.05$).

2.4 Effects of Dietary Protein Level on Digestive Enzyme Activities

As shown in Table 5 , dietary protein level significantly affected intestinal and hepatic protease and amylase activities ($P<0.05$), but not lipase activity ($P>0.05$). Intestinal protease activity increased significantly with dietary protein level, reaching the highest value in the 50.53% group, significantly higher than the first five groups ($P<0.05$). Intestinal amylase activity decreased significantly ($P<0.05$), with the lowest value in the 50.53% group, significantly lower than the first five groups ($P<0.05$). Hepatic digestive enzyme activities showed similar trends: protease activity was highest in the 50.53% group, with the 40.26%, 45.42%, and 50.53% groups significantly higher than the 25.63% group ($P<0.05$); amylase activity was lowest in the 50.53% group, significantly lower than all groups except 45.42% ($P<0.05$). Hepatic and intestinal lipase activities were not significantly affected by dietary protein level ($P>0.05$).

3.1 Effects of Dietary Protein Level on Growth Performance and Feed Utilization

The survival rate of 99.05–100% across all groups indicated that juvenile hybrid F1 adapted well to the experimental conditions and diets, with their docile temperament contributing to high survival. WGR and SGR increased significantly when dietary protein increased from 25.63% to 35.13%, demonstrating that dietary protein level significantly affected growth performance. Growth performance plateaued after 35.13% protein, with no significant differences among the 35.13–50.53% groups. PER and PPV also declined after 45.42% protein, indicating that excessive protein was not used for growth but likely catabolized for energy. Similar results have been reported for grass carp, Taiwan loach, and Japanese flounder, where growth performance plateaued and PER decreased significantly beyond optimal protein levels.

VSI and HSI decreased with increasing dietary protein level, likely because the iso-lipidic and iso-energetic formulation resulted in higher carbohydrate content in low-protein diets. Excess carbohydrate intake in fish leads to lipid deposition in liver and visceral adipose tissue, increasing VSI and HSI. Similar trends were observed in Erythroculter ilishaeformis, Taiwan loach, starry flounder, and Dabry' s sturgeon. However, VSI and HSI were unaffected by dietary protein level in yellow drum and northern stone loach, possibly due to differences in species, diet formulation, and experimental conditions.

The broken-line model is appropriate for analyzing nutrient requirements when growth performance increases linearly then stabilizes. Based on WGR and PER, the dietary protein requirement for juvenile hybrid F1 was 36.43–38.81%, higher than the 32% observed in our previous pond demonstrations. This discrepancy likely reflects differences in life stage and culture environment. Juveniles have faster growth rates and higher metabolic demands, increasing protein requirements. Additionally, natural food availability in pond culture may reduce dietary protein requirements. The requirement for hybrid F1 juveniles (36.43–

38.81%) was lower than reported for Erythroculter ilishaeformis juveniles (40.94–41.35%). While Erythroculter ilishaeformis is carnivorous with high protein requirements, hybrid F1 grows faster than both parents. The significant reduction in PER at 40.26–50.53% protein suggests that hybrid F1 may reduce protein requirements by improving protein utilization efficiency.

3.2 Effects of Dietary Protein Level on Whole-Body Composition

Whole-body crude protein increased with dietary protein level from 25.63% to 40.26%, but remained consistent at 40.26–50.53%. This pattern, similar to findings in Japanese flounder and starry flounder, suggests that excess dietary protein was not deposited in tissues but primarily oxidized for energy, leading to decreased PPV at high protein levels. However, dietary protein level did not significantly affect whole-body protein content in northern stone loach or Nile tilapia, likely due to species and experimental differences.

Whole-body crude lipid decreased with increasing dietary protein level, reaching the lowest value at 50.53% protein, consistent with results for Erythroculter ilishaeformis and Japanese sea bass. Although dietary lipid levels were similar across groups, low-protein diets contained higher carbohydrate content. Excess carbohydrate intake in fish is converted to lipid, increasing whole-body lipid content. Whole-body moisture (69.95–71.25%) and ash content (3.01–3.28%) were unaffected by dietary protein level, consistent with findings for Erythroculter ilishaeformis, Nile tilapia, and Japanese sea bass.

3.3 Effects of Dietary Protein Level on Serum Biochemical Indices

Serum biochemical indices reflect physiological status and nutrient metabolism, providing indicators of fish health and nutritional condition. TG and TCHO, major blood lipids synthesized primarily in the liver, reflect lipid metabolism. The lack of significant differences in serum TG and TCHO among groups aligns with findings for Nile tilapia, Dabry' s sturgeon, and beluga sturgeon, likely due to consistent dietary lipid levels.

Serum TP content reflects nutritional status and protein digestion/absorption, being significantly influenced by dietary protein level. The initial increase followed by decrease in serum TP at 40.26% protein indicates that protein exceeding physiological requirements was not effectively absorbed and was wasted, mirroring trends in PER and growth performance. AST and ALT are important aminotransferases catalyzing amino acid-keto acid transamination and participating in amino acid biosynthesis. The general increase in serum AST and ALT activities with dietary protein level is consistent with findings in Dabry' s sturgeon.

3.4 Effects of Dietary Protein Level on Digestive Enzyme Activities

Intestinal protease activity in juvenile hybrid F1 increased significantly with dietary protein level, peaking at 50.53% protein, similar to results for Erythrocul-

ter ilishaeformis, yellow drum, and Japanese sea bass. As the primary digestive organ, the intestine is sensitive to dietary protein changes, with dietary protein stimulating protease secretion. However, some studies suggest that excessive protein may increase digestive burden and negatively regulate intestinal protease secretion through feedback inhibition. In Spinibarbus caldwelli, intestinal amylase activity did not continue increasing but decreased significantly when dietary protein reached 40.26–50.53%. In this study, hepatic protease activity increased initially then stabilized at 40.26–50.53% protein, indicating asynchronous adaptive responses among organs and differing trends in intestinal versus hepatic digestive enzyme activities.

Intestinal and hepatic amylase activities showed opposite trends to protease, decreasing with increasing dietary protein level and reaching the lowest values at 50.53% protein, consistent with dietary dextrin levels and indicating that amylase activity was primarily influenced by carbohydrate content. Most studies show that lipase activity in cultured fish is unaffected by dietary protein level, as reported for Erythroculter ilishaeformis, rohu, and yellow drum. The lack of significant effects on intestinal and hepatic lipase activity in this study aligns with previous findings, likely due to consistent dietary lipid levels across groups.

4 Conclusion

Under the experimental conditions, broken-line regression analysis based on weight gain rate and protein efficiency ratio indicated that the dietary protein requirement of juvenile Erythroculter ilishaeformis ( ) × Anchorythroculter nigrocauda ( ) hybrid F1 was 36.43% and 38.81%, respectively.

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