Effects of Dietary Protein Level on Growth Performance, Morphometric Indices, and Body Composition of Taiwan Loach (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary protein levels on growth performance, morphological indices, and body composition of juvenile Taiwan loach. A total of 720 juvenile Taiwan loach with an initial body weight of (8.57$±$0.35) g were randomly assigned to 4 groups with 3 replicates per group (60 fish per replicate) and fed experimental diets containing 25%, 30%, 35%, and 40% protein for 60 days. The results showed that final body weight and weight gain rate of Taiwan loach increased initially and then stabilized with increasing dietary protein levels, reaching maxima at 35% dietary protein. Feed conversion ratio decreased initially and then stabilized with increasing dietary protein levels, reaching its minimum at 40% dietary protein. No significant differences were observed in final body weight, weight gain rate, or feed conversion ratio when dietary protein level increased from 35% to 40% ($P > 0.05$). Broken-line model analysis based on weight gain rate and feed conversion ratio indicated that the dietary protein levels for maximum weight gain rate and minimum feed conversion ratio in Taiwan loach were 34.57% and 35.47%, respectively. Protein efficiency ratio, protein retention rate, and survival rate increased initially and then decreased with increasing dietary protein levels. Quadratic polynomial regression analysis revealed that the dietary protein levels for maximum protein efficiency ratio and protein retention rate were 33.61% and 34.68%, respectively. Viscera-somatic index and hepatosomatic index decreased initially and then stabilized with increasing dietary protein levels. Crude protein content in fish body increased initially and then stabilized, while crude lipid content decreased initially and then stabilized with increasing dietary protein levels. These results suggest that moderately increasing dietary protein levels can enhance fish growth rate, increase the proportion of edible parts, and improve protein efficiency ratio and protein retention rate; however, excessively high dietary protein

levels reduce protein retention rate and protein efficiency ratio. Under the experimental conditions of this study, the optimal dietary protein level for Taiwan loach is 33.61%-35.47% when considering growth performance indices and protein utilization comprehensively.

Full Text

Effects of Dietary Protein Level on Growth Performance, Physical Indices and Body Composition of Taiwan Loach

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Abstract

This study aimed to investigate the effects of dietary protein level on growth performance, physical indices, and body composition of juvenile Taiwan loach (*Paramisgurnus* sp.). A total of 720 fish with an initial body weight of (8.57$±$0.35) g were randomly divided into 4 groups with 3 replicates per group and 60 fish per replicate. Four experimental diets containing protein levels of 25%, 30%, 35%, and 40% were fed to the fish for 60 days. The results showed that with increasing dietary protein level, final weight (FW) and weight gain rate (WGR) of Taiwan loach initially increased and then plateaued, reaching maximum values at 35% dietary protein. Feed conversion ratio (FCR) initially decreased and then plateaued with increasing dietary protein level, with the lowest value observed at 40% dietary protein. No significant changes were observed in FW, WGR, or FCR when dietary protein level increased from 35% to 40% (P>0.05). Broken-line analysis based on WGR and FCR indicated that the optimal dietary protein level for maximum WGR was 34.57%, while the level for minimum FCR was 35.47%. Protein efficiency ratio (PER), protein retention efficiency (PRE), and survival rate (SR) all initially increased and then decreased with rising dietary protein level. Quadratic polynomial regression analysis revealed that the dietary protein levels for maximum PER and PRE were 33.61% and 34.68%, respectively. Viscerasomatic index (VSI) and hepaticsomatic index (HSI) initially decreased and then plateaued with increasing dietary protein level. Whole-body crude protein content initially increased and then plateaued, while whole-body crude lipid content initially

decreased and then plateaued. These results suggest that moderately increasing dietary protein level can enhance fish growth rate, increase the proportion of edible parts, and improve PER and PRE. However, excessively high dietary protein levels reduce PRE and PER. Under the experimental conditions, considering both growth performance and protein utilization, the suitable dietary protein level for Taiwan loach is 33.61%–35.47%.

Keywords: Taiwan loach; protein level; growth performance; physical indices; body composition

Introduction

In aquaculture feeds, protein is an essential core nutrient and relatively the most expensive ingredient. Dietary protein level directly affects both feed cost and fish growth. When dietary protein is insufficient, fish cannot achieve optimal growth rates [1] and survival rate may be compromised [2]. Conversely, excessive dietary protein is catabolized through oxidative deamination for energy production, resulting in increased ammonia excretion that pollutes the culture water, while also raising feed costs—both detrimental to fish growth and ecological sustainability [3-5]. To ensure profitable aquaculture, dietary protein levels in formulated feeds must be appropriately set according to the actual protein requirements of the cultured species. To date, protein requirements have been established for the loach *Misgurnus anguillicaudatus* [6-7].

Taiwan loach is an improved variety of loach developed in Taiwan Province, China, belonging to Cypriniformes, Cobitidae, Cobitinae, and genus *Paramisgurnus*, though its exact taxonomic status remains unclear [8-9]. Taiwan loach offers several advantages including rapid growth, strong disease resistance, high stocking density, large individual size, short culture period, and ease of harvest [10], making it popular among farmers and consumers. In recent years, Taiwan loach has been introduced to mainland China and is now widely cultured. By 2014, the culture area in Guangdong Province had reached 10,000 mu (1 mu 667 m^2) [11]. As the scale of Taiwan loach aquaculture expands, demands for high-quality formulated feeds continue to increase. Current research on Taiwan loach has primarily focused on artificial propagation [9,12], early development [9], and morphology [13]. However, no studies have reported on the nutrient requirements of Taiwan loach, particularly regarding dietary protein. This experiment aimed to determine the optimal dietary protein requirement for Taiwan loach and investigate how different protein levels affect growth performance, physical indices, and body composition, thereby providing a theoretical basis for scientifically formulated feeds.

Materials and Methods

1.1 Experimental Diets

Fish meal, soybean meal, and rapeseed meal were used as protein sources, soybean oil as the lipid source, and corn meal and wheat middling as carbohydrate sources. Four iso-lipidic and iso-energetic experimental diets were formulated with protein levels of 25%, 30%, 35%, and 40%. Feed ingredients were ground to pass through a 60-mesh sieve, weighed according to formulation, and thoroughly mixed. The powdered mixture was stored at -4°C until use. The composition and nutrient levels of the experimental diets are presented in Table 1 .

1.2 Experimental Design and Culture Management

Juvenile Taiwan loach were purchased from Rongchang Xiangguang Loach Farm and represented the same batch of propagated fish. Upon arrival, fish were disinfected with 3% salt solution and acclimated in temporary holding tanks (1.2 m × 0.5 m × 0.8 m) for 7 days. A total of 720 healthy, uninjured fish with an initial body weight of (8.57$±$0.35) g were randomly allocated into 4 groups with 3 replicates per group and 60 fish per replicate. Fish were stocked in 12 concrete tanks (1.2 m × 0.5 m × 0.8 m) and fed one of the four experimental diets with different protein levels. Fish were hand-fed to apparent satiation three times daily (07:00, 12:00, 17:00) at a feeding rate of 3–5% of body weight. One hour after feeding, uneaten feed and feces were collected by siphoning, dried, and weighed. The experiment lasted for 60 days. During the culture period, one-third of the water was exchanged daily with aerated tap water between 17:00 and 19:00. Water quality parameters, temperature, mortality, and feeding behavior were monitored daily. Water temperature was maintained at 20.0–24.0°C, pH at 7.0–7.5, and dissolved oxygen at $ $6.0 mg/L.

1.3 Sample Collection and Index Determination

At the end of the experiment, fish were fasted for 24 h before counting and weighing. Ten fish were randomly selected from each group, anesthetized with 50 mg/L MS-222 solution, and measured for body length and weight. Viscera and hepatopancreas were then dissected and weighed to calculate hepaticsomatic index (HSI) and viscerasomatic index (VSI). Additionally, five fish from each group were randomly sampled and stored at -20°C for body composition analysis. Proximate composition analysis of diets and whole fish, muscle, and hepatopancreas followed AOAC (1995) methods [14]. Crude protein content was determined by the Kjeldahl method, crude lipid content by Soxhlet extraction (using ether as solvent), moisture content by oven drying at 105°C to constant weight, and crude ash content by carbonization on an electric furnace followed by combustion in a muffle furnace at 550°C for 12 h.

1.4 Calculation Formulas

The following formulas were used for calculations: Weight gain rate (WGR, %) = 100 × (Wt - W0) / W0; Feed conversion ratio (FCR) = F / (Wt - W0); Protein retention efficiency (PRE, %) = 100 × (Wt × BP2 - W0 × BP1) / (F × P); Protein efficiency ratio (PER) = (Wt - W0) / (F × P); Survival rate (SR, %) = 100 × Nf / Ni; Condition factor (CF, g/cm^3) = Wt / L^3; Viscerasomatic index (VSI, %) = 100 × Wv / Wt; Hepaticsomatic index (HSI, %) = 100 × Wh / Wt. Where W0 and Wt represent initial and final body weight (g), respectively; F is feed intake (g); BP1 and BP2 are initial and final whole-body crude protein content (%), respectively; P is dietary crude protein content (%); Ni and Nf are the number of fish at the beginning and end of the experiment, respectively; L is body length (cm); Wv and Wh are the weights of viscera and liver (g), respectively.

1.5 Statistical Analysis

Results are expressed as mean ± standard deviation (SD). One-way ANOVA was performed using SPSS 19.0 statistical software. When significant differences were detected, Duncan' s multiple range test was used for post-hoc comparisons. The significance level was set at $P < 0.05$.

Results

2.1 Effects of Dietary Protein Level on Growth Performance of Taiwan Loach

The effects of dietary protein level on growth performance of Taiwan loach are presented in Table 2 . Final weight and weight gain rate initially increased and then plateaued with increasing dietary protein level, reaching maximum values of 31.22 g and 259.87% at 35% dietary protein. No significant changes were observed in final weight or weight gain rate when dietary protein level increased from 35% to 40% ($P > 0.05$). Protein efficiency ratio, protein retention efficiency, and survival rate all initially increased and then decreased, reaching maximum values of 2.77, 44.73%, and 98.89% at 35% dietary protein, respectively. Feed conversion ratio initially decreased and then plateaued with increasing dietary protein level, with the lowest value of 0.99 observed at 40% dietary protein. No significant difference in FCR was found when dietary protein level increased from 35% to 40% ($P > 0.05$). Broken-line analysis based on weight gain rate and feed conversion ratio (Fig. 1 [Figure 1: see original paper], Fig. 2 [Figure 2: see original paper]) indicated that the dietary protein level for maximum weight gain rate was 34.57%, while the level for minimum FCR was 35.47%. Quadratic regression analysis of dietary protein level (X) versus protein efficiency ratio (Y1) and protein retention efficiency (Y2) (Fig. 3 [Figure 3: see original paper], Fig. 4 [Figure 4: see original paper]) yielded the equations: $Y1 = -38X^2 + 25.54X - 1.583$ ($R^2 = 0.8094$) and $Y2 = -724X^2 + 502.12X - 43.669$ ($R^2 = 0.8636$). The dietary protein levels for maximum PER and PRE were calculated to be 33.61%

and 34.68%, respectively.

2.2 Effects of Dietary Protein Level on Physical Indices of Taiwan Loach

The effects of dietary protein level on physical indices of Taiwan loach are shown in Table 3 . Both viscerasomatic index and hepaticsomatic index initially decreased and then plateaued with increasing dietary protein level. VSI reached its lowest value of 6.50% at 40% dietary protein, while HSI reached its minimum of 2.54% at 35% dietary protein. No significant changes in VSI or HSI were observed when dietary protein level increased from 35% to 40% ($P > 0.05$). No significant differences in condition factor were detected among all groups ($P > 0.05$).

2.3 Effects of Dietary Protein Level on Body Composition of Taiwan Loach

The effects of dietary protein level on body composition of Taiwan loach are presented in Table 4 . Whole-body crude protein content initially increased and then plateaued with increasing dietary protein level, reaching the highest value of 16.15% at 35% dietary protein. Whole-body crude lipid content initially decreased and then plateaued, with the lowest value of 4.50% observed at 40% dietary protein. No significant differences in crude protein or crude lipid content were found when dietary protein level increased from 35% to 40% ($P > 0.05$). Moisture and crude ash contents remained unchanged across all dietary protein levels ($P > 0.05$).

Discussion

3.1 Effects of Dietary Protein Level on Growth Performance of Taiwan Loach

Dietary protein level is a critical factor affecting fish growth. Increasing protein level generally improves fish growth, but excessive protein is deaminated and utilized for energy, which significantly increases feed costs given the high price of protein sources [15]. In this experiment, final weight, weight gain rate, protein retention efficiency, and protein efficiency ratio of Taiwan loach showed strong correlations with dietary protein level. With increasing dietary protein level, final weight and weight gain rate initially increased and then plateaued, showing little change when dietary protein level reached 40%. Li et al. [16] reported similar findings in grass carp (*Ctenopharyngodon idellus*), where excessively high dietary protein was not fully utilized for growth. Two possible reasons explain this phenomenon: First, protein is a vital component of fish tissue; moderately increasing dietary protein meets nutritional requirements and promotes growth, but once the optimal level is reached, excess protein is catabolized for energy. Second, since the experimental diets were iso-energetic, high-protein diets contained less non-protein energy, forcing large amounts of protein to be used as

an energy source [17], thereby eliminating any further growth-promoting effects. Similar results were reported by Salhi et al. [18] for jundiá (*Rhamdia quelen*) and Chen et al. [19] for grass carp. However, Papaparaskeva-Papoutsoglou et al. [20] for flathead grey mullet (*Mugil capito*), Tibbetts et al. [21] for American eel (*Anguilla rostrata*), and Chou et al. [22] for cobia (*Rachycentron canadum*) found that dietary protein levels exceeding a certain threshold reduced fish growth performance, which differs from our results. These discrepancies may be attributed to differences in fish species, size, and dietary energy levels. Evidently, increasing dietary protein within a certain range promotes growth and reduces feed conversion ratio, but beyond this range, protein retention efficiency and protein efficiency ratio decrease. Additionally, excessive protein catabolism produces large amounts of ammonia, polluting the water, while the high cost of protein ingredients increases feed expenses. Based on broken-line analysis of weight gain rate and feed conversion ratio, and quadratic regression of protein retention efficiency and protein efficiency ratio, the optimal dietary protein level for Taiwan loach with an average weight of 8.57 g was determined to be 33.61%–35.47%. Ye et al. [6] reported that the optimal dietary protein level for *Misgurnus anguillicaudatus* (1.72 g) was 45.5%, while Shen et al. [7] found the optimal level to be 39.55% for 4.7 g loach cultured for 8 weeks. The optimal dietary protein level for Taiwan loach in our study was significantly lower than that for *Misgurnus anguillicaudatus* reported by Ye et al. [6] and Shen et al. [7], possibly due to differences in species, fish size, and culture temperature. Li et al. [16] demonstrated that protein requirement decreases with increasing fish size in grass carp. Chen et al. [23] showed that···

3.2 Effects of Dietary Protein Level on Physical Indices of Taiwan Loach

Some studies have reported that dietary protein level showed no significant effect on hepaticsomatic index and viscerasomatic index, which differs from our results. This discrepancy may be related to fish species and dietary nutrient composition, and the specific reasons require further investigation. Our results indicate that feeding Taiwan loach high-protein diets can reduce the proportions of viscera and liver, thereby increasing the proportion of edible parts.

3.3 Effects of Dietary Protein Level on Body Composition of Taiwan Loach

Most studies have found that increasing dietary protein level gradually elevates whole-body crude protein content while reducing crude lipid content [22,30-31]. Tian et al. [32] for grass carp and Chen et al. [33] for Japanese sea bass (*Lateolabrax japonicus*) reported that whole-body crude protein content increased significantly with dietary protein level. Chen et al. [34] found that whole-body crude lipid content was higher in black carp (*Mylopharngodon piceus*) fed low-protein diets. In this experiment, whole-body crude protein content of Taiwan loach initially increased and then plateaued with increasing dietary protein level,

while crude lipid content initially decreased and then plateaued, consistent with the aforementioned studies. As dietary protein level increased, protein intake also increased, and more digested and absorbed protein could be utilized as building blocks for tissue repair and new tissue formation [35]. Ma et al. [36] reported that in Amur catfish (*Pseudobagrus ussuriensis*), low dietary protein levels resulted in high energy-to-protein ratios, which enhanced hepatic lipogenic enzyme activity. This caused unconsumed carbohydrates to be converted to fat in hepatocytes and transported for storage, promoting lipid deposition. Therefore, moderately increasing dietary protein level can increase whole-body crude protein content while reducing crude lipid content.

Based on the comprehensive results of this study, three key conclusions can be drawn: First, protein is a fundamental component of fish tissue, and moderately increasing dietary protein meets nutritional requirements, promotes growth, reduces viscerasomatic index, increases edible parts, and enhances protein intake. Digested and absorbed protein serves as building blocks for tissue, thereby improving protein retention efficiency and whole-body crude protein content. Second, excessively high dietary protein levels reduce protein retention efficiency and protein efficiency ratio. Third, under the experimental conditions and considering both growth performance and protein utilization, the suitable dietary protein level for Taiwan loach is 33.61%–35.47%.

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Figure Captions

Fig. 1 Effects of dietary protein level on weight gain rate (WGR) of Taiwan loach
Fig. 2 Effects of dietary protein level on feed conversion ratio (FCR) of Taiwan loach
Fig. 3 Effects of dietary protein level on protein retention efficiency (PRE) of Taiwan loach
Fig. 4 Effects of dietary protein level on protein efficiency ratio (PER) of Taiwan loach

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