

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

**Authors:** Zeng Benhe, Wang Wanliang, Zhu Long, Chen Meiqun, Liu Haiping, Zhang Chi, Mou Zhenbo, Zhu Chengke, Xiang Xiao, Zhou Jianshe

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

### Abstract

This experiment aimed to explore the effects of dietary protein levels on growth performance, morphometric indices, and body composition of juvenile Taiwan loach. A total of 720 juvenile Taiwan loach with an initial body weight of  $(8.57 \pm 0.35)$  g were selected and randomly divided into 4 groups with 3 replicates per group and 60 fish per replicate, fed experimental diets with protein levels of 25%, 30%, 35%, and 40% for a culture period of 60 days. The results showed that with increasing dietary protein levels, final body weight and weight gain rate of Taiwan loach both exhibited a trend of first increasing and then stabilizing, reaching maximum values at a dietary protein level of 35%. Feed conversion ratio showed a trend of first decreasing and then stabilizing with increasing dietary protein levels, reaching its lowest value at a dietary protein level of 40%. When dietary protein level increased from 35% to 40%, there were no significant changes in final body weight, weight gain rate, and feed conversion ratio ( $P > 0.05$ ). Based on broken-line regression analysis of weight gain rate and feed conversion ratio, the dietary protein level for maximum weight gain rate of Taiwan loach was 34.57%, and the dietary protein level for lowest feed conversion ratio was 35.47%. With increasing dietary protein levels, protein efficiency ratio, protein retention rate, and survival rate all exhibited a trend of first increasing and then decreasing. Through quadratic polynomial regression analysis, the dietary protein levels for maximum protein efficiency ratio and protein retention rate of Taiwan loach were 33.61% and 34.68%, respectively. With increasing dietary protein levels, viscera-somatic index and hepatosomatic index of Taiwan loach both exhibited a trend of first decreasing and then stabilizing, crude protein content in fish body showed a trend of first increasing and then stabilizing, and crude lipid content in fish body showed a trend of first decreasing and then stabilizing. The results suggest that appropriately increasing dietary protein level can improve fish growth rate, increase

the proportion of edible parts, and enhance protein efficiency ratio and protein retention rate; however, excessively high dietary protein levels will reduce protein retention rate and protein efficiency ratio. Under the conditions of this experiment, considering growth performance indices and protein utilization comprehensively, the suitable dietary protein level for Taiwan loach was 33.61%–35.47%.

## Full Text

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

ZENG Benhe<sup>1,2</sup>, WANG Wanliang<sup>1</sup>, ZHU Long<sup>2</sup>, CHEN Meiqun<sup>1</sup>, LIU Haiping<sup>1</sup>, ZHANG Chi<sup>1</sup>, MOU Zhenbo<sup>1</sup>, ZHU Chengke<sup>2</sup>, XIANG Xiao<sup>2</sup>, ZHOU Jianshe<sup>1\*</sup>

<sup>1</sup>Institute of Fisheries Science, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850002, China

<sup>2</sup>Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, Key Laboratory of Aquatic Science of Chongqing, Department of Fisheries, Rongchang Campus, Southwest University, Chongqing 402460, China

## Abstract

This experiment was conducted to investigate the effects of dietary protein level on growth performance, physical indices, and body composition of juvenile Taiwan loach. A total of 720 fish with an initial body weight of  $(8.57 \pm 0.35)$  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 respective groups for 60 days. The results showed that final weight and weight gain rate of Taiwan loach initially increased and then plateaued with increasing dietary protein level, reaching maximum values at 35% dietary protein. Feed conversion ratio decreased initially and then stabilized with increasing dietary protein level, attaining its lowest value at 40% dietary protein. No significant changes were observed in final 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 level for maximum weight gain rate was 34.57%, while the level for minimum feed conversion ratio was 35.47%. Protein efficiency ratio, protein retention efficiency, and survival rate all increased initially and then decreased with rising dietary protein level. Quadratic polynomial regression analysis revealed that dietary protein levels for maximum protein efficiency ratio and protein retention efficiency were 33.61% and 34.68%, respectively. Viscerosomatic index and hepatosomatic index decreased initially and then stabilized with increasing dietary protein level. Whole-body crude protein content increased initially and then plateaued, while whole-body crude lipid content decreased initially and then stabilized.

These results suggest that appropriately increasing dietary protein level can improve fish growth rate, increase the proportion of edible body parts, and enhance protein efficiency ratio and protein retention efficiency. However, excessively high dietary protein levels reduce protein retention efficiency and protein efficiency ratio. Under the experimental conditions, the suitable dietary protein level for Taiwan loach is 33.61%–35.47% based on comprehensive consideration of growth performance indicators and protein utilization.

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

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## Introduction

Protein is an essential core nutrient and relatively the most expensive ingredient in aquatic animal feeds. Dietary protein level directly affects both feed product price and fish growth. When dietary protein level is insufficient, fish cannot achieve optimal growth rates and survival rate may be compromised. Conversely, when dietary protein level is excessive, fish metabolize surplus protein through oxidative deamination for energy expenditure, producing substantial ammonia nitrogen excretions that pollute aquaculture water while increasing feed costs—both detrimental to fish growth and ecological sustainability. Therefore, to ensure favorable aquaculture economic benefits, protein levels in artificial compound feeds must be reasonably established according to the actual protein requirements of cultured fish species.

Currently, researchers have determined protein requirements for loach (*Misgurnus anguillicaudatus*). Taiwan loach is a new improved loach variety from Taiwan Province, China, belonging to Cypriniformes, Cobitidae, Cobitinae, and *Paramisgurnus* genus, though its specific taxonomic status remains unclear. Taiwan loach offers advantages including rapid growth, strong disease resistance, high stocking density, large individual size, short culture cycle, and easy harvesting, making it popular among farmers and consumers. In recent years, Taiwan loach has been introduced to mainland China and is being cultured in various regions. By 2014, Taiwan loach aquaculture area in Guangdong Province had reached 10,000 mu (1 mu = 667 m<sup>2</sup>). With expanding cultivation scale, quality requirements for compound feeds continue to increase. Current research on Taiwan loach has primarily focused on artificial reproduction, early development, and morphology, while studies on dietary nutrient requirements remain unreported, resulting in a lack of nutritional data. This experiment aims to determine the optimal dietary protein requirement for Taiwan loach and investigate the effects of different protein levels on growth performance, physical indices, and body composition, providing a theoretical basis for scientific formulation of artificial compound feeds.

### 1.1 Experimental Diets

Fish meal, soybean meal, and rapeseed meal served as protein sources, soybean oil as 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 mixed uniformly. The powdered diets were stored at  $-4^{\circ}\text{C}$  until use. Dietary composition and nutrient levels are presented in Table 1 .

### 1.2 Experimental Design and Management

Juvenile Taiwan loach were purchased from Rongchang Xiangguang Loach Farm as a single batch of propagated fish. After disinfection with 3% salt water, fish were temporarily cultured in holding tanks ( $1.2\text{ m} \times 0.5\text{ m} \times 0.8\text{ m}$ ) for 7 days. A total of 720 healthy, uninjured fish with body weight of ( $8.57 \pm 0.35$ ) g were randomly allocated into 4 groups with 3 replicates per group and 60 fish per replicate, and stocked in 12 concrete tanks ( $1.2\text{ m} \times 0.5\text{ m} \times 0.8\text{ m}$ ). Each group was 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 3-5% of body weight. After 1 hour of feeding, residual feed and feces were collected by siphoning, dried, and weighed. The experiment lasted 60 days. During the culture period, one-third of the water was exchanged daily with aerated tap water between 17:00-19:00. Water temperature, pH, dissolved oxygen, fish mortality, and feeding behavior were monitored daily. Water temperature was maintained at  $20.0\text{-}24.0^{\circ}\text{C}$ , pH at 7.0-7.5, and dissolved oxygen 6.0 mg/L.

### 1.3 Sample Collection and Index Determination

At the end of the experiment, fish were fasted for 24 h, then counted and weighed. 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 dissected and weighed to calculate hepatosomatic index (HSI) and viscerasomatic index (VSI). Additionally, five fish from each group were randomly sampled and stored at  $-20^{\circ}\text{C}$  for body composition analysis.

Contents of moisture, crude protein, crude lipid, and crude ash in diets, whole fish, muscle, and hepatopancreas were determined according to AOAC (1995) methods. Crude protein content was measured by the Kjeldahl method, crude lipid by Soxhlet extraction (using ether as solvent), moisture by atmospheric drying at  $105^{\circ}\text{C}$  to constant weight, and crude ash by carbonization on an electric furnace followed by combustion at  $550^{\circ}\text{C}$  for 12 h.

### 1.4 Calculation Formulas

Weight gain rate (WGR, %) =  $100 \times (W_t - W_0) / W_0$

Feed conversion ratio (FCR) =  $F / (W_t - W_0)$

Protein retention efficiency (PRE, %) =  $100 \times (W_t \times BP_2 - W_0 \times BP_1) / (F \times P)$

Protein efficiency ratio (PER) =  $(W_t - W_0) / (F \times P)$

Survival rate (%) =  $100 \times N_f / N_i$

Condition factor (CF, g/cm<sup>3</sup>) =  $W_t / L^3$

Viscerasomatic index (%) =  $100 \times W_v / W_t$

Hepatosomatic index (%) =  $100 \times W_h / W_t$

Where:  $W_0$  and  $W_t$  are initial and final body weight (g);  $F$  is feed intake (g);  $BP_1$  and  $BP_2$  are initial and final whole-body crude protein content (%);  $P$  is dietary crude protein content (%);  $N_f$  and  $N_i$  are final and initial fish number;  $L$  is body length (cm);  $W_v$  and  $W_h$  are viscera and liver weight (g).

### 1.5 Statistical Analysis

Results are expressed as mean  $\pm$  standard deviation (SD). One-way ANOVA was performed using SPSS 19.0 statistical software. When significant differences were detected, Duncan's multiple comparison test was applied. Significance level was set at  $P < 0.05$ .

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

The effects of dietary protein level on growth performance are shown in Table 2. Final weight and weight gain rate of Taiwan loach 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 increased initially and then decreased, attaining maximum values of 2.77, 44.73%, and 98.89% at 35% dietary protein, respectively. Feed conversion ratio decreased initially and then stabilized with increasing dietary protein level, reaching its lowest value of 0.99 at 40% dietary protein. No significant change in feed conversion ratio was observed 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 (Figure 1 [Figure 1: see original paper], Figure 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 feed conversion ratio was 35.47%. Quadratic curve fitting of dietary protein level ( $X$ ) against protein efficiency ratio ( $Y_1$ ) and protein retention efficiency ( $Y_2$ ) (Figure 3 [Figure 3: see original paper], Figure 4 [Figure 4: see original paper]) yielded regression equations:  $Y_1 = -38X^2 + 25.54X - 1.583$  ( $R^2 = 0.8094$ ) and  $Y_2 = -724X^2 + 502.12X - 43.669$  ( $R^2 = 0.8636$ ). The dietary protein levels for maximum protein efficiency ratio

and protein retention efficiency were 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 are presented in Table 3. Viscerasomatic index and hepatosomatic index of Taiwan loach decreased initially and then stabilized with increasing dietary protein level. Viscerasomatic index reached its lowest value of 6.50% at 40% dietary protein, while hepatosomatic index reached its lowest value of 2.54% at 35% dietary protein. No significant changes were observed in viscerasomatic index or hepatosomatic index when dietary protein level increased from 35% to 40% ( $P>0.05$ ). No significant differences in condition factor were detected among 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 are shown in Table 4. Whole-body crude protein content of Taiwan loach increased initially and then plateaued with increasing dietary protein level, reaching its highest value of 16.15% at 35% dietary protein. Whole-body crude lipid content decreased initially and then stabilized with increasing dietary protein level, reaching its lowest value of 4.50% at 40% dietary protein. No significant changes were observed in crude protein or crude lipid content when dietary protein level increased from 35% to 40% ( $P>0.05$ ). Moisture and crude ash contents showed no significant changes across dietary protein levels ( $P>0.05$ ).

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## 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 as energy, substantially increasing feed costs due to the high price of protein sources. 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. Final weight and weight gain rate increased initially and then plateaued with rising dietary protein level, showing little change when dietary protein level further increased to 40%. Similar findings were reported in grass carp (*Ctenopharyngodon idellus*) by Li et al., who found that excessively high dietary protein level was not fully utilized for fish growth. This may be explained by two factors: First, protein is an essential body component, and appropriate increases in dietary protein meet nutritional requirements and promote growth; once protein requirements are satisfied, surplus protein is catabolized for energy. Second, since experimental diets were iso-energetic, high-protein diets contained less non-protein energy, resulting in

substantial protein catabolism for energy utilization at high protein levels, thus eliminating growth-promoting effects. Similar results were reported in *Rhamdia quelen* by Salhi et al. and in grass carp by Chen et al.

However, some studies showed that dietary protein levels exceeding certain thresholds reduced fish growth performance, including research on mullet (*Mugil capito*) by Papaparaskeva-Papoutsoglou et al., American eel (*Anguilla rostrata*) by Tibbetts et al., and cobia (*Rachycentron canadum*) by Chou et al., which differed from our results. These discrepancies may be related to fish species, size, dietary energy levels, and other factors. Evidently, increasing dietary protein within a certain range promotes fish growth and reduces feed conversion ratio, but excessive protein decreases protein retention efficiency and protein efficiency ratio, generates substantial ammonia nitrogen metabolites that pollute water, and increases feed costs due to expensive protein ingredients.

Broken-line modeling of weight gain rate and feed conversion ratio, combined with quadratic regression of protein retention efficiency and protein efficiency ratio, indicated that the optimal dietary protein level for Taiwan loach with average weight of 8.57 g was 33.61%-35.47%. Ye et al. reported that the optimal dietary protein level for growth of loach (*Misgurnus anguillicaudatus*) with average weight of 1.72 g was 45.5%. Shen et al. found that the optimal dietary protein level for 4.7 g loach was 39.55% after 8 weeks. The optimal dietary protein level for Taiwan loach in our study was significantly lower than these reported values for loach, possibly due to differences in fish species, size, and culture temperature. Li et al. demonstrated that protein requirements of grass carp decreased with fish growth. Chen et al. showed that the suitable dietary protein level for 1.5 g grouper (*Epinephelus malabaricus*) was 54%, decreasing to 40%-50% for 20-30 g fish. Studies have also indicated that fish protein requirements increase with water temperature; for instance, protein requirements of red drum (*Sciaenops ocellatus*) were 35% and 44% at water temperatures of 22-26°C and 26-33°C, respectively.

In this experiment, broken-line modeling suggested that dietary protein levels for maximum growth rate and minimum feed conversion ratio were 34.57%-35.47%, while quadratic regression indicated that dietary protein levels for maximum protein retention efficiency and protein efficiency ratio were 33.61%-34.68%. The overlapping range demonstrates that appropriate dietary protein not only enhances growth performance but also improves protein retention efficiency and whole-body crude protein content.

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

In this experiment, viscerasomatic index and hepatosomatic index of Taiwan loach decreased with increasing dietary protein level. Similar results were reported in juvenile starry flounder by Ding et al. When fish consume low-protein diets containing high carbohydrate levels, the activity of lipid synthesis enzymes

in certain tissues increases, promoting conversion of carbohydrate sources to lipids that are transported and stored in liver and abdominal adipose tissues, thereby increasing viscera and liver weights. However, studies on yellow drum (*Nibea albiflora*) by Qiu et al. and Nile tilapia (*Oreochromis niloticus*) by Yang et al. found no significant effects of dietary protein level on hepatosomatic index or viscerasomatic index, differing from our results. These discrepancies may be related to fish species and dietary nutrient composition, requiring further investigation. Our results indicate that high-protein diets reduce viscera and liver proportions, increasing the edible portion of Taiwan loach.

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

Most studies have shown that increasing dietary protein level gradually elevates whole-body crude protein content while decreasing crude lipid content. Research on grass carp by Tian et al. and Japanese seabass (*Lateolabrax japonicus*) by Chen et al. demonstrated that whole-body crude protein content increased significantly with dietary protein level. Chen et al. reported that whole-body crude lipid content was higher in black carp (*Mylopharyngodon piceus*) fed low-protein diets. In our experiment, whole-body crude protein content of Taiwan loach increased initially and then plateaued with rising dietary protein level, while crude lipid content decreased initially and then stabilized, consistent with aforementioned studies. As dietary protein level increased, protein intake and digestion/absorption increased, providing more building proteins for tissue repair and new tissue formation. Ma et al. found that low dietary protein levels resulted in high energy-to-protein ratios, which enhanced activity of lipid synthesis enzymes in liver, promoting conversion of unconsumed carbohydrates to lipids that were transported and stored extracellularly, thereby facilitating lipid deposition. Therefore, appropriate increases in dietary protein level can elevate whole-body crude protein content and reduce crude lipid content.

In conclusion, protein is an essential body component, and appropriate increases in dietary protein meet nutritional requirements, promote growth, reduce viscerasomatic index, increase edible portion, enhance protein intake and utilization as building proteins, thereby improving protein retention efficiency and whole-body crude protein content. However, excessively high dietary protein levels reduce protein retention efficiency and protein efficiency ratio. Under the experimental conditions, the suitable dietary protein level for Taiwan loach is 33.61%-35.47% based on comprehensive consideration of growth performance and protein utilization.

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