

## Effects of Dietary Lipid Level on Growth Performance, Muscle Composition, and Plasma Biochemical Indices of Juvenile Yellow Drum (*Nibea albiflora*): Postprint

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### Abstract

This study used fish oil as the primary lipid source to formulate four isonitrogenous diets with lipid levels of 4.07% (L4 group), 7.04% (L7 group), 10.05% (L10 group), and 12.97% (L13 group). A total of 1,200 juvenile yellow drum (*Nibea albiflora*) with an initial average body weight of  $(41.7 \pm 3.1)$  g were divided into four groups and subjected to an 8-week feeding trial to investigate the effects of dietary lipid levels on growth performance, muscle composition, and plasma biochemical indices of juvenile yellow drum. Each group had three replicates, with 100 fish stocked per replicate. The results showed that the final average weight, final average body length, weight gain rate, survival rate, feed efficiency, protein efficiency ratio, condition factor, and viscera-somatic index of juvenile yellow drum in the L4 and L7 groups were significantly higher than those in the L10 and L13 groups ( $P < 0.05$ ). The specific growth rate and hepatosomatic index of juvenile yellow drum in the L7 group were significantly higher than those in the L10 and L13 groups ( $P < 0.05$ ), but no significant differences were observed between the L7 and L4 groups ( $P > 0.05$ ). The muscle crude protein content of juvenile yellow drum in the L4, L7, and L10 groups was significantly higher than that in the L13 group ( $P < 0.05$ ), with no significant differences among the L4, L7, and L10 groups ( $P > 0.05$ ). The muscle crude lipid content of juvenile yellow drum in the L4 group was significantly lower than that in the L7, L10, and L13 groups ( $P < 0.05$ ), with no significant differences among the L7, L10, and L13 groups ( $P > 0.05$ ). The muscle crude ash content of juvenile yellow drum in the L7 group was significantly lower than that in the L4, L10, and L13 groups ( $P < 0.05$ ), with no significant differences among the L4, L10, and L13 groups ( $P > 0.05$ ). The plasma total protein, albumin, globulin, triglyceride, high-density lipoprotein, and low-density lipoprotein contents of juvenile yellow drum in the L4 and L7 groups were significantly higher than those in the L10

and L13 groups ( $P < 0.05$ ), whereas the glucose and cholesterol contents and the activities of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase were significantly lower than those in the L10 and L13 groups ( $P < 0.05$ ). Based on these experimental results, under the conditions of this study, the optimal dietary lipid level for juvenile yellow drum is 7.04%.

## Full Text

### Effects of Dietary Lipid Level on Growth Performance, Muscle Composition and Plasma Biochemical Indices of Juvenile *Nibea albiflora*

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## Abstract

This study investigated the effects of dietary lipid level on growth performance, muscle composition, and plasma biochemical indices of juvenile *Nibea albiflora*. Four iso-nitrogenous diets were formulated with fish oil as the primary lipid source, containing lipid levels of 4.07% (L4 group), 7.04% (L7 group), 10.05% (L10 group), and 12.97% (L13 group). A total of 1,200 juvenile *Nibea albiflora* with an initial average body weight of ( $41.7 \pm 3.1$ ) g were divided into four groups and fed the experimental diets for eight weeks. Each group consisted of three replicates with 100 fish per replicate. The results demonstrated that the final average weight, final average length, weight gain rate, survival rate, feed efficiency, protein efficiency ratio, condition factor, and viserosomatic index of fish in the L4 and L7 groups were significantly higher than those in the L10 and L13 groups ( $P < 0.05$ ). The specific growth rate and hepatosomatic index in the L7 group were significantly higher than in the L10 and L13 groups ( $P < 0.05$ ), though no significant differences were observed between the L4 and L7 groups ( $P > 0.05$ ). Muscle crude protein content in the L4, L7, and L10 groups was significantly higher than in the L13 group ( $P < 0.05$ ), with no significant differences among the first three groups ( $P > 0.05$ ). Muscle crude lipid content in the L4 group was significantly lower than in the L7, L10, and L13 groups ( $P < 0.05$ ), while no significant differences were found among the latter three groups ( $P > 0.05$ ). Muscle ash content in the L7 group was significantly lower than in the L4, L10,

and L13 groups ( $P < 0.05$ ), with no significant differences among these three groups ( $P > 0.05$ ). Plasma total protein, albumin, globulin, triglyceride, high-density lipoprotein, and low-density lipoprotein levels in the L4 and L7 groups were significantly higher than in the L10 and L13 groups ( $P < 0.05$ ), whereas plasma glucose and cholesterol concentrations and lactate dehydrogenase, alanine transaminase, aspartate transaminase, and alkaline phosphatase activities were significantly lower ( $P < 0.05$ ). Based on these findings, the optimal dietary lipid level for juvenile *Nibea albiflora* under the present experimental conditions is 7.04%.

**Keywords:** juvenile *Nibea albiflora*; dietary lipid level; growth performance; muscle composition; plasma biochemical indices

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## Introduction

*Nibea albiflora* (Richardson) belongs to the class Osteichthyes, order Perciformes, family Sciaenidae, and genus *Nibea*. This warm-temperate, nearshore, demersal fish species is primarily distributed in the coastal waters of the Korean Peninsula, southern Japan, and China, representing an important economic fishery resource [1-3]. Valued for its delicious taste and nutritional quality, *Nibea albiflora* is particularly prized for its swim bladder, which is used in traditional Chinese medicine. The species exhibits strong resistance to *Cryptocaryon irritans* infection (white spot disease) and maintains high survival rates during later culture stages, making it increasingly popular among aquaculturists and one of the rapidly emerging cultured species in Fujian and Zhejiang provinces in recent years.

Previous research has extensively investigated the reproductive biology [4-10], artificial culture techniques [11-14], and disease management [15-18] of *Nibea albiflora*, establishing a solid foundation for industry development. However, studies on nutritional requirements remain limited [19-21], and no specialized formulated feeds are currently available on the market. Most farmers continue to use frozen trash fish or generic marine fish feeds, which present significant challenges. The inconsistent quality of frozen trash fish and the resulting water pollution contribute to frequent disease outbreaks, while commercial marine fish feeds often fail to meet the specific nutritional requirements of *Nibea albiflora*, leading to slow growth and high feed costs that are unacceptable to producers. Therefore, developing efficient and environmentally friendly formulated feeds specifically for *Nibea albiflora* is urgently needed to support the industrial development of its aquaculture.

Lipids are essential components of fish tissues, providing energy, essential fatty acids, and serving as dissolution media for fat-soluble vitamins and certain hormones [22-24]. They play a crucial role in the growth and reproduction of aquatic animals and represent a key parameter in formulated feed development. Both excessive and insufficient dietary lipid levels can negatively impact fish

growth and health, making the provision of optimal lipid levels critical in feed formulation.

This study employed a single-factor experimental design to investigate the effects of different dietary lipid levels on growth performance, body composition, and plasma biochemical indices of juvenile *Nibea albiflora*, providing a theoretical basis for healthy aquaculture practices and rational lipid supplementation in formulated feeds for this species.

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## Materials and Methods

### Experimental Fish

Juvenile *Nibea albiflora* were provided by Ningde Hengyudao Aquatic Co., Ltd. Over 1,500 fish with an average weight of approximately 40 g were initially selected and acclimated indoors in 10 m<sup>3</sup> cement tanks for one week before the experiment commenced.

### Experimental Diets

Casein and white fish meal served as the primary protein sources, with fish oil as the lipid source. Based on preliminary trial results, the dietary protein level was set at 40%, and four iso-nitrogenous diets were formulated with target lipid levels of 4% (L4), 7% (L7), 10% (L10), and 13% (L13). The measured lipid levels were 4.07%, 7.04%, 10.05%, and 12.97%, respectively. Dietary composition and nutrient levels are presented in . Feed ingredients were ground and passed through a 60-mesh sieve, weighed according to formula proportions, and mixed thoroughly in a blender. Appropriate amounts of water were added during mixing to form a dough, which was then extruded through a meat grinder into pellets (5-8 mm length, 3 mm diameter). Pellet size was adjusted according to the growth stage of the juvenile fish. Prepared feeds were stored at 4 °C and removed 0.5 h before feeding to return to room temperature.

### Experimental Design

After one week of indoor acclimation, 1,200 healthy juvenile *Nibea albiflora* with an initial average body weight of (41.7±3.1) g were selected and randomly divided into four groups, each with three replicates of 100 fish. Fish were stocked in cement tanks measuring 2 m × 1 m × 1 m. Each group was fed one of the four iso-nitrogenous diets containing 4.07% (L4), 7.04% (L7), 10.05% (L10), or 12.97% (L13) lipid. The water depth was maintained at 0.8 m, with aeration provided by air stones. The feeding trial lasted for eight weeks.

## Culture Management

Fish were fed three times daily (08:00, 13:00, and 18:00) at a rate of 3-6% of body weight, adjusted according to weather conditions, feeding response, and fish health status. Uneaten feed was siphoned 0.5 h after each feeding, freeze-dried, and weighed to calculate feed intake. Tank cleaning and water exchange (100% daily) were performed after morning and evening feedings. Daily records were maintained for feeding behavior, health status, and mortality, with dead fish removed promptly to prevent water quality deterioration. Culture water was sand-filtered natural seawater maintained at  $(27 \pm 2)$  °C, pH  $8.0 \pm 0.5$ , salinity 27-29, dissolved oxygen  $>5$  mg/L, ammonia nitrogen  $<0.05$  mg/L, and nitrite  $<0.05$  mg/L.

## Sample Collection

**Feed Samples** Using the quartering method, 50 g of feed was collected from each experimental group, placed in self-sealing bags, and stored at -20 °C.

**Fish Samples** At the end of the trial, fish were fasted for 24 h before counting and weighing. Body weight and length were measured for each fish. Subsequently, five fish per tank were anesthetized with eugenol, and blood was collected from the caudal vein into 2 mL centrifuge tubes containing 1% heparin sodium to prepare anticoagulant samples. Blood samples were centrifuged at 4,000 r/min for 10 min, and the supernatant was collected and refrigerated at -4 °C for analysis. After blood collection, fish were dissected to remove viscera including the liver for calculation of hepatosomatic and viserosomatic indices. Muscle samples were collected in self-sealing bags and stored at -80 °C for nutrient composition analysis.

## Growth Indices and Calculation Formulas

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

Specific growth rate (SGR, %/d) =  $100 \times (\ln W_t - \ln W_0) / t$

Feed efficiency (FE, %) =  $100 \times (W_t - W_0) / W_d$

Protein efficiency ratio (PER, %) =  $100 \times (W_t - W_0) / W_p$

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

Condition factor (CF, %) =  $100 \times W_b / L^3$

Hepatosomatic index (HSI, %) =  $100 \times W_h / W_b$

Viserosomatic index (VSI, %) =  $100 \times W_e / W_b$

Where:  $W_t$  = final body weight (g);  $W_0$  = initial body weight (g);  $t$  = feeding days (d);  $W_b$  = individual body weight (g);  $W_d$  = total feed intake (g);  $W_p$  = total protein intake (g);  $L$  = individual body length (cm);  $W_h$  = liver weight (g);  $N_i$  = initial fish number;  $N_f$  = final fish number;  $W_e$  = viscera weight (g).

### **Nutrient Composition Analysis**

Crude protein content was determined using an automatic Kjeldahl nitrogen analyzer (FOSS KT260, Switzerland). Crude lipid content was measured using a Soxhlet extraction apparatus (FOSS Soxtec-2055, Sweden). Moisture content was determined by oven drying at 105 °C. Ash content was measured by muffle furnace incineration at 550 °C.

### **Plasma Biochemical Indices Determination**

Plasma samples were thawed and centrifuged at 3,000 r/min for 10 min to remove gelatinous substances. Biochemical indices were measured using an automatic biochemical analyzer (Selectra XL, Netherlands), including total protein (TP), albumin (ALB), globulin (GLB), alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), glucose (GLU), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), chloride (Cl<sup>-</sup>), calcium (Ca<sup>2+</sup>), triglyceride (TG), cholesterol (CHOL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and lactate dehydrogenase (LDH).

### **Data Analysis**

Experimental data are expressed as mean  $\pm$  standard deviation (mean  $\pm$  SD). Statistical analysis was performed using ANOVA in SPSS 13.0 software. When significant differences were detected ( $P < 0.05$ ), Tukey's test was used for multiple comparisons.

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## **Results**

### **Effects of Dietary Lipid Level on Growth Performance of Juvenile *Nibea albiflora***

As shown in , the final average weight, final average length, survival rate, feed efficiency, condition factor, and viserosomatic index of juvenile *Nibea albiflora* in the L4 and L7 groups were significantly higher than those in the L10 and L13 groups ( $P < 0.05$ ), with no significant differences between the L4 and L7 groups ( $P > 0.05$ ), though the L7 group showed slightly higher values. The specific growth rate and hepatosomatic index in the L7 group were significantly higher than in the L10 and L13 groups ( $P < 0.05$ ), with no significant difference between the L7 and L4 groups ( $P > 0.05$ ), though the L7 group was slightly higher. The protein efficiency ratio and viserosomatic index in the L7 group were significantly higher than in all other groups ( $P < 0.05$ ), while the L4 group showed significantly higher values than the L10 and L13 groups ( $P < 0.05$ ).

### **Effects of Dietary Lipid Level on Muscle Composition of Juvenile *Nibea albiflora***

As shown in , the muscle crude protein content of juvenile *Nibea albiflora* in the L4, L7, and L10 groups was significantly higher than in the L13 group ( $P < 0.05$ ), with no significant differences among the first three groups ( $P > 0.05$ ), though the L7 group was slightly higher than the L4 and L10 groups. Muscle crude lipid content in the L4 group was significantly lower than in the L7, L10, and L13 groups ( $P < 0.05$ ), while no significant differences were observed among the latter three groups ( $P > 0.05$ ). Muscle ash content in the L7 group was significantly lower than in the L4, L10, and L13 groups ( $P < 0.05$ ), with no significant differences among these three groups ( $P > 0.05$ ). No significant differences in muscle moisture content were detected among all experimental groups ( $P > 0.05$ ).

### **Effects of Dietary Lipid Level on Plasma Biochemical Indices of Juvenile *Nibea albiflora***

As shown in , plasma TP, ALB, GLB, TG, CHOL, HDL, and LDL contents in juvenile *Nibea albiflora* showed a gradual decreasing trend with increasing dietary lipid levels, while GLU content and LDH, ALT, AST, and ALP activities exhibited a gradual increasing trend. The TP, ALB, GLB, and TG contents in the L4 and L7 groups were significantly higher than in the L10 and L13 groups ( $P < 0.05$ ), whereas GLU and CHOL contents and LDH, ALT, AST, and ALP activities were significantly lower ( $P < 0.05$ ). HDL and LDL contents in the L4 group were significantly higher than in the L10 and L13 groups ( $P < 0.05$ ). No significant differences were observed in plasma Na, K, Cl, and Ca<sup>2+</sup> contents among all groups ( $P > 0.05$ ).

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## **Discussion**

### **Effects of Dietary Lipid Level on Growth Performance of Juvenile *Nibea albiflora***

Lipids serve as a crucial energy source for fish life activities and are indispensable for growth and development. Appropriate dietary lipid levels can accelerate fish growth, improve feed utilization, and conserve protein [25]. The present results indicate that moderately increasing dietary lipid level (from 4.07% to 7.04% in this study) significantly promoted growth, enhanced feed efficiency, and reduced nitrogen excretion in juvenile *Nibea albiflora*, demonstrating a “protein-sparing effect” [26]. However, excessive lipid levels significantly reduced growth rate and feed efficiency. The L10 and L13 groups exhibited significantly lower key growth indices and feed efficiency compared to the L7 and/or L4 groups. During the trial, fish in the L10 and L13 groups displayed characteristics of reduced feed intake, decreased activity, darkened body color, and emaciation—

symptoms similar to those observed in fatty liver disease reported in rainbow trout (*Oncorhynchus mykiss*) [27], red drum (*Sciaenops ocellatus*) [28], and Nile tilapia (*Oreochromis niloticus* × *O. aureus*) [29]. Additionally, survival rates decreased significantly, suggesting that fish in these groups may have developed fatty liver disease with severely compromised health and viability, whereas the L7 and L4 groups maintained higher survival rates.

Yi et al. [30] reported that high-lipid diets promoted growth in juvenile large yellow croaker (*Larimichthys croceus*, 10 g body weight), with an optimal dietary lipid level of 11.7%. Huang et al. [31] found that the optimal lipid level for *Nibea coibor* was 12.9%. These discrepancies may be attributed to differences in fish species, size, dietary ingredient composition, and culture environment. The present study utilized diets with 40% crude protein based on preliminary trials indicating this level as appropriate for juvenile *Nibea albiflora*, which aligns with the findings of Qiu et al. [32] but differs substantially from Lu et al. [21], possibly due to variations in fish size, stocking density, and environmental conditions. Under the given dietary protein level, this study preliminarily determined that the optimal dietary lipid level for juvenile *Nibea albiflora* is 7.04%.

### **Effects of Dietary Lipid Level on Muscle Composition of Juvenile *Nibea albiflora***

Protein and lipid content are commonly used indicators for evaluating the nutritional value and quality of fish muscle [33]. Dietary lipids are digested in the intestine by lipases into fatty acids, which either enter various tissues directly via blood or are transported through the hepatic portal vein to the liver for resynthesis of lipids before distribution to muscle and other tissues. When dietary lipid levels are excessive, hepatic lipid synthesis increases, leading to greater lipid deposition in various tissues. However, the liver's transport capacity is limited, and excessive dietary lipids cannot be efficiently exported, resulting in elevated body lipid content, energy surplus, reduced nutrient utilization efficiency, and impaired protein absorption and deposition in muscle and other tissues [25].

The present results showed no significant differences in muscle crude protein content among the L4, L7, and L10 groups. However, when dietary lipid level increased to 12.97% for eight weeks, muscle crude protein content decreased significantly compared to the other three groups, accompanied by significantly lower protein efficiency ratio. This indicates that excessive dietary lipid levels are detrimental to dietary protein conversion and protein deposition in fish. Muscle crude lipid content increased with rising dietary lipid levels, demonstrating that surplus dietary lipids were deposited in fish tissues. However, once dietary lipid level reached a certain threshold (above 10.04%), muscle crude lipid content stabilized, consistent with findings in European sea bass (*Dicentrarchus labrax*) reported by Peres et al. [34].

## Effects of Dietary Lipid Level on Plasma Biochemical Indices of Juvenile *Nibeia albiflora*

Blood biochemical indices reflect fish physiological metabolism and are closely related to nutritional status. Plasma TP and ALP contents are important indicators of protein absorption and metabolism, GLU content reflects carbohydrate absorption, transport, and metabolism, TG is involved in energy production and storage, and CHOL participates in cell membrane formation and serves as a precursor for bile acid, vitamin D, and steroid hormone synthesis [25]. This study found that plasma TP, ALB, GLB, TG, and CHOL contents in juvenile *Nibeia albiflora* decreased gradually with increasing dietary lipid levels, declining significantly when lipid level reached 10.05%. This may be attributed to abnormal bile secretion for lipid digestion or hepatic dysfunction, hindering TG export from the liver and causing rapid reduction in blood TG content. These results suggest that high-lipid diets may cause liver function impairment or hepatocyte damage, leading to adverse physiological metabolic conditions in juvenile *Nibeia albiflora*, consistent with findings by Wang et al. [35] and Shi et al. [36]. However, studies by Wang et al. [37], Kjaer et al. [38], and Dong et al. [39] reported positive correlations between serum CHOL content and dietary lipid level, contradicting our results. Some studies have also indicated that fish may develop fatty liver even at lipid levels suitable for growth or lower, possibly related to dietary energy, protein levels, and phospholipid content [40-41], warranting further investigation.

In this study, plasma ALT and AST activities increased continuously with rising dietary lipid levels, with significant differences among all groups. ALT and AST participate in transamination processes and are primarily distributed in skeletal muscle, liver, and kidney tissues. Their activity levels are closely associated with hepatocyte inflammation, degeneration, and necrosis, serving as sensitive indicators of liver cell damage [42]. Under normal conditions, only small amounts enter the bloodstream. When tissue damage increases cell membrane permeability, osmoregulatory capacity and active transport mechanisms are compromised or lost, leading to cell disintegration and release of ALT and AST into the blood, thereby increasing their activities [43-44]. HDL and LDL are responsible for lipid metabolism, and their blood levels decrease significantly when liver function is abnormal or pathological. This study showed that plasma HDL and LDL contents decreased gradually with increasing dietary lipid levels, possibly due to pathological changes caused by excessive lipid levels [29]. ALP is related to nutritional immunity and normally exhibits low activity, but increases significantly when liver and other tissues become pathological. LDH is a marker enzyme for anaerobic metabolism, and its blood activity rises rapidly when tissue damage causes intracellular LDH release [45]. In this study, plasma HDL and LDL contents and ALT, AST, ALP, and LDH activities increased significantly when dietary lipid level reached 12.97%, indicating that excessive lipid levels may cause liver and other tissue damage, leading to release of cellular components into the blood and significantly elevated enzyme activities. Dietary

lipid level had minimal effects on plasma Na , K , Cl , and Ca<sup>2</sup> contents.

Under the conditions of this study, growth performance, protein efficiency ratio, and feed efficiency declined when dietary lipid level exceeded 7.04%, while plasma liver function indicators ALT and AST activities increased. Based on these comprehensive indices, the optimal dietary lipid level for juvenile *Nibea albiflora* is 7.04%.

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