Effects of Different Diets on Growth Performance, Body Composition, and Digestive Enzyme Activity in Juvenile Chinese Giant Salamanders (Andrias davidianus): Postprint

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Date: 2017-11-08T00:00:00+00:00

Abstract

To determine the optimal feed for giant salamander larvae, this experiment investigated the effects of different feeds on growth performance, body composition, and digestive enzyme activity of giant salamander larvae. The experiment selected 300 giant salamander larvae with an average body weight of (1.08$±$0.01) g, which were randomly divided into 5 groups with 3 replicates per group and 20 individuals per replicate. The five groups of giant salamander larvae were fed five different feeds, namely fish flesh, mealworm, aquatic earthworm, brine shrimp, and river shrimp, with a culture period of 60 d. The results showed that different feeds had no significant effects on survival rate, whole-body moisture, or crude ash content of giant salamander larvae ($P>0.05$); the river shrimp group exhibited the highest final mean weight, weight gain rate, and specific growth rate among all groups, which were significantly higher than those of the aquatic earthworm and brine shrimp groups ($P<0.05$); the condition factor of the mealworm and aquatic earthworm groups was significantly higher than that of the brine shrimp group ($P<0.05$). Dry matter efficiency was significantly higher in the fish flesh and aquatic earthworm groups than in the other groups ($P<0.05$), significantly higher in the river shrimp group than in the brine shrimp and mealworm groups ($P<0.05$), and significantly higher in the brine shrimp group than in the mealworm group ($P<0.05$); protein efficiency differed significantly among groups ($P<0.05$), showing the trend: aquatic earthworm group > river shrimp group > fish flesh group > brine shrimp group > mealworm group; lipid efficiency was significantly higher in the fish flesh group than in the other groups ($P<0.05$), significantly higher in the brine shrimp and river shrimp groups than in the aquatic earthworm and mealworm groups ($P<0.05$), and significantly higher in the aquatic earthworm group than in the mealworm group (P<0.05). The river shrimp group exhibited the highest whole-body crude protein content among all groups, which was significantly higher than that of the mealworm and brine shrimp groups (P<0.05); meanwhile, the mealworm group showed the highest whole-body crude lipid content, which was significantly higher than that of all other groups (P<0.05). There were no significant differences among groups in the contents of 17 amino acids, total amino acids, total essential amino acids, or total flavor amino acids in whole-body of giant salamander larvae (P>0.05); however, the ratio of essential to non-essential amino acids was significantly higher in the aquatic earthworm, brine shrimp, and river shrimp groups than in the fish flesh and mealworm groups (P<0.05). Gastrointestinal protease activity in giant salamander larvae was significantly higher in the fish flesh and river shrimp groups than in the mealworm and brine shrimp groups (P<0.05); gastrointestinal lipase activity was significantly higher in the mealworm group than in all other groups (P<0.05); gastrointestinal amylase activity was significantly lower only in the brine shrimp group compared to the other groups (P<0.05). In summary, river shrimp is the optimal feed for giant salamander larvae, followed by fish flesh, with aquatic earthworm and mealworm being the least recommended options.

Full Text

Effects of Different Diets on Growth Performance, Body Composition and Digestive Enzyme Activities of Juvenile Chinese Giant Salamander (Andrias davidianus)

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Abstract

To determine the optimal diet for juvenile Chinese giant salamander, this study investigated the effects of different diets on growth performance, body composition, and digestive enzyme activities. Three hundred juvenile Chinese giant salamanders with an average body weight of (1.08$±$0.01) g were randomly allocated into five groups, each with three replicates of 20 individuals. The five groups were fed five different diets: raw fish block, mealworm (*Tenebrio molitor*), tubifex, brine shrimp, and caridina for 60 days. The results showed that different diets had no significant effects on survival rate or whole-body moisture and ash content (P>0.05). The caridina group exhibited the highest final body weight, weight gain rate, and specific growth rate, which were significantly higher than those of the tubifex and brine shrimp groups (P<0.05). The condition factor of juveniles in the mealworm and tubifex groups was significantly higher than that in the brine shrimp group (P<0.05). Dry matter efficiency was significantly higher in the raw fish block and tubifex groups compared to other groups (P<0.05), while the caridina group was significantly higher than the brine shrimp and mealworm groups (P<0.05), and the brine shrimp group was significantly higher than the mealworm group (P<0.05). Protein efficiency ratio differed significantly among groups (P<0.05), following the pattern: tubifex > caridina > raw fish block > brine shrimp > mealworm. Lipid efficiency ratio in the raw fish block group was significantly higher than in other groups (P<0.05), while the brine shrimp and caridina groups were significantly higher than the tubifex and mealworm groups (P<0.05), and the tubifex group was significantly higher than the mealworm group (P<0.05). Whole-body crude protein content was highest in the caridina group, significantly higher than in the mealworm and brine shrimp groups (P<0.05), whereas whole-body crude fat content was highest in the mealworm group, significantly higher than in all other groups (P<0.05).

No significant differences were observed among groups in the contents of 17 amino acids, total amino acids, essential amino acids, or delicious amino acids in whole-body composition (P>0.05). However, the essential amino acids to non-essential amino acids ratio in the tubifex, brine shrimp, and caridina groups was significantly higher than in the raw fish block and mealworm groups (P<0.05). Protease activity in the gastrointestinal tract was significantly higher in the raw fish block and caridina groups compared to the mealworm and brine shrimp groups (P<0.05), while lipase activity was significantly higher in the mealworm group than in all other groups (P<0.05). Amylase activity was significantly lower in the brine shrimp group compared to other groups (P<0.05). In conclusion, caridina represents the optimal diet for juvenile Chinese giant salamander, followed by raw fish block, with tubifex and mealworm being less suitable options.

Keywords: Chinese giant salamander; different diets; growth performance; body composition; digestive enzymes

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The Chinese giant salamander (*Andrias davidianus*), commonly known as the "baby fish," belongs to the class Amphibia, order Urodela, and family Cryptobranchidae. It is the largest extant amphibian species and a rare "living fossil" that coexisted with dinosaurs 300 million years ago [1]. As a precious national specialty of China, it is listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) and classified as a second-class national protected wild animal [2]. The salamander' s tender meat and unique flavor confer high economic, medicinal, and edible value [3]. Since China' s first successful artificial breeding in 1978, nearly 30 years of research and promotion have led to the gradual development of large-scale and intensive aquaculture, which offers substantially higher benefits than traditional farming [4-5].

Currently, Chinese giant salamander aquaculture primarily relies on fresh fish and shrimp, with no better alternative feeds identified. The high cost of feed has become a major bottleneck constraining the healthy development of the industry [6-7]. As aquaculture scales expand, researchers have investigated feed types and feeding rates. Yang et al. [8] reported that salamander larvae prefer animal-based feeds over plant-based feeds, with small aquatic insect larvae promoting the fastest growth, followed by minced fish and shrimp, while minced pork, beef, and mutton are also consumed but can deteriorate water quality. Li et al. [9] found that chironomid larvae and small fish/shrimp are suitable feeds for one-year-old salamanders (~0.7 g). Ouyang et al. [10] suggested that tubifex is more suitable than artificial diets for larval stages. However, these studies determined optimal feeds based solely on weight gain rate, lacking comprehensive and systematic investigation. Similar studies on malabar grouper (*Epinephelus malabaricus*) [11], paddlefish (*Polyodon spathula*) [12], and Chinese sucker (*Myxocyprinus asiaticus*) [13] have identified optimal feeds through multiple aspects including growth performance and digestive enzyme activities.

Building on previous research, this experiment selected five natural diets for juvenile Chinese giant salamander: raw fish block, mealworm (*Tenebrio molitor*), tubifex, brine shrimp, and caridina. By examining the effects of these diets on growth performance, body composition, and digestive enzyme activities, this study aims to explore suitable feeds and nutritional requirements for juvenile salamanders, providing reference for developing artificial formulated feeds.

1.1 Experimental Animals and Diets

Juvenile Chinese giant salamanders used in this experiment were F2 generation individuals artificially bred at the Jurong Longquan Chinese Giant Salamander Propagation Center. Three hundred healthy juveniles with uniform size and an average body weight of (1.08$±$0.01) g were randomly distributed into 15 plastic boxes (40 cm × 25 cm × 15 cm), with 20 individuals per box. The five experimental groups were fed raw fish block, mealworm, tubifex, brine shrimp, and caridina, respectively. Tubifex and brine shrimp were frozen products purchased from the market, mealworms were live insects purchased from the market and cut to appropriate size before feeding, caridina were naturally produced within the farm' s water system, and raw fish block was prepared from small trash fish cultured in the farm' s feed ponds. Nutritional components of the different diets are presented in Table 1 , and amino acid composition is shown in Table 2 . Throughout the experiment, the feeding rate was maintained at 3%-4% of body weight, and all diets were disinfected with 2% saline solution for 20 minutes before feeding.

1.2 Feeding Management

The feeding trial was conducted at the Jurong Longquan Chinese Giant Salamander Propagation Center. Each group was acclimated to the respective diet for 10 days before the experiment began. Culture water was natural spring water from the center' s cave, supplied directly to the culture tanks via pipelines. Water temperature remained at (19$±2)°*Cyear* −

$round, dissolved oxygen concentration was (6.0\pm1.0) mg/L, and pH was 7.5\pm$0.3, all meeting national standards for freshwater aquaculture. During the culture period, water depth was maintained at 1.0-3.0 cm with flow-through culture. Feeding occurred once daily at 15:00, with water flow stopped before feeding. The following day at 08:00, feeding activity was observed, residual feed was removed, and water flow was resumed. The experimental period lasted 60 days.

1.3 Index Determination and Methods

On days 20 and 40 of the experiment, salamanders in each box were counted and weighed as a group. At the end of the trial, after 24 hours of fasting, salamanders were counted and weighed as a group, and 10 individuals were randomly selected for individual weight and length measurements to calculate survival rate (SR), weight gain ratio (WGR), specific growth rate (SGR), and condition factor (CF). Additionally, six salamanders were collected from each box: three were dissected to obtain stomach and intestinal tissues, which were stored at -20°C for digestive enzyme activity analysis, while the remaining three were used for determination of whole-body moisture, crude protein, crude fat, ash content, and amino acid composition. Determination methods for moisture, crude protein, and ash content in feeds and fish bodies followed AOAC (1990) [14]. Moisture content was determined by weight loss after drying at 105°C to constant weight. Crude protein content was measured using a Kjeldahl analyzer (2300 Kjeltec Analyzer Unit, FOSS TECATOR, Sweden). Ash content was determined by weight loss after combustion in a muffle furnace at 550°C. Crude fat content in feeds and fish bodies was determined according to Folch et al. [15]. Amino acid composition was analyzed using an amino acid analyzer (Hitachi L-8900, Japan) after hydrochloric acid hydrolysis. Gastrointestinal protease activity was measured using the Folin-phenol reagent method [16], lipase activity by chemical turbidimetry, and amylase activity by iodine-starch colorimetry. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4 Calculation Formulas

Weight gain ratio (%) = 100 × (final average weight - initial average weight) / initial average weight

Specific growth rate (%/d) = 100 × (ln final average weight - ln initial average weight) / culture days

Condition factor (g/cm^3) = 100 × body weight / body length^3

Survival rate (%) = 100 × number of surviving juveniles / number of experimental juveniles

Dry matter efficiency ratio (DMER, %) = 100 × weight gain / dry matter intake

Protein efficiency ratio (PER, %) = 100 × weight gain / (feed intake × crude protein content in feed)

Lipid efficiency ratio (LER, %) = 100 × weight gain / (feed intake × crude fat content in feed)

1.5 Data Statistics and Analysis

Raw data were initially organized using Excel 2003. SPSS 18.0 was used for one-way ANOVA, and Duncan' s multiple range test was applied to examine significant differences among groups, with $P<0.05$ considered statistically significant. Data are expressed as mean ± standard error (mean±SE).

2.1 Effects of Different Diets on Growth Performance and Feed Conversion Efficiency

As shown in Table 3 , no significant differences in survival rate were observed among groups ($P>0.05$), and no tissue lesions were visually detected. However, abdominal distension was observed in some mealworm group juveniles, with a few individuals floating on the water surface. The caridina group exhibited the highest final body weight, final body length, weight gain rate, and specific growth rate, which were significantly higher than those of the tubifex and brine shrimp groups ($P<0.05$) but not significantly different from the mealworm and raw fish block groups ($P>0.05$). Condition factor of juveniles in the mealworm and tubifex groups was significantly higher than that in the brine shrimp group ($P<0.05$), with no significant differences from the other two groups ($P>0.05$).

Figure 1 [Figure 1: see original paper] shows that on day 20 of the experiment, average body weight in the mealworm group was highest (2.04 g), significantly exceeding that of the tubifex, brine shrimp, and caridina groups ($P<0.05$). By day 40, average body weight in the tubifex and brine shrimp groups was significantly lower than in the other three groups ($P<0.05$), while the caridina and raw fish block groups had surpassed the mealworm group, though not significantly ($P>0.05$).

As shown in Table 4 , dry matter intake in the mealworm group was significantly higher than in all other groups ($P<0.05$), followed by the caridina group, which was significantly higher than the remaining three groups ($P<0.05$). The raw fish block group was significantly higher than the tubifex and brine shrimp groups ($P<0.05$). Dry matter efficiency was significantly higher in the raw fish block and tubifex groups compared to other groups ($P<0.05$), with the caridina group significantly higher than the brine shrimp and mealworm groups ($P<0.05$), and the brine shrimp group significantly higher than the mealworm group ($P<0.05$). Significant differences in protein intake and protein efficiency ratio were observed among groups ($P<0.05$). Protein intake followed the pattern: mealworm > raw fish block > caridina > brine shrimp > tubifex, while protein efficiency ratio followed: tubifex > caridina > raw fish block > brine shrimp > mealworm. Lipid intake was highest in the mealworm group, significantly exceeding all other groups ($P<0.05$), followed by the caridina and tubifex groups, which were significantly higher than the raw fish block and brine shrimp groups ($P<0.05$). Lipid efficiency ratio in the raw fish block group was significantly higher than in all other groups ($P<0.05$), while the brine shrimp and caridina groups were significantly higher than the tubifex and mealworm groups ($P<0.05$), and the tubifex group was significantly higher than the mealworm group (P<0.05).

2.2 Effects of Different Diets on Whole-Body Conventional Nutritional Components and Amino Acid Composition

As shown in Table 5 , whole-body crude protein content was highest in the caridina group (13.46%), significantly higher than in the mealworm and brine shrimp groups (P<0.05) but not significantly different from the raw fish block and tubifex groups (P>0.05). The raw fish block, tubifex, and mealworm groups were significantly higher than the brine shrimp group (P<0.05). Whole-body crude fat content was highest in the mealworm group (3.81), significantly exceeding all other groups (P<0.05), while the tubifex group had the lowest crude fat content (3.12), significantly lower than the raw fish block and caridina groups (P<0.05) but not significantly different from the brine shrimp group (P>0.05). No significant differences in whole-body moisture or ash content were observed among groups (P>0.05).

No significant differences were found among groups in the contents of 17 amino acids, total amino acids, essential amino acids, or delicious amino acids in whole-body composition (P>0.05). However, the essential amino acids to non-essential amino acids ratio in the tubifex, brine shrimp, and caridina groups was significantly higher than in the raw fish block and mealworm groups (P<0.05).

2.3 Effects of Different Diets on Gastrointestinal Digestive Enzyme Activities

As shown in Table 7 , protease activity in the gastrointestinal tract was significantly higher in the raw fish block and caridina groups compared to the mealworm and brine shrimp groups (P<0.05), with no significant difference from the tubifex group (P>0.05), though the tubifex group was significantly higher than the brine shrimp group (P<0.05). Lipase activity was significantly higher in the mealworm group than in all other groups (P<0.05), while the brine shrimp group exhibited the lowest lipase activity (15.12), significantly lower than the raw fish block, tubifex, and caridina groups (P<0.05). Amylase activity was lowest in the brine shrimp group (0.22), significantly lower than in the raw fish block and caridina groups (P<0.05), but not significantly different from the mealworm and tubifex groups (P>0.05).

3.1 Effects of Different Diets on Growth Performance and Feed Conversion Efficiency

The growth and development of fish larvae and juveniles are closely related to diet type, size (palatability), density (availability), and nutritional composition after initial feeding [12]. Wild Chinese giant salamanders primarily consume animal-based diets, with juveniles feeding on small invertebrates such as tubifex, water fleas, small fish and shrimp, and aquatic insect larvae [2]. Previous studies suggest that small aquatic insect larvae (e.g., chironomid larvae) are superior to small fish and shrimp as feed for salamander larvae, while pork, beef, and mutton can deteriorate water quality and are unsuitable [8-9]. Other research indicates that tubifex is more appropriate than artificial diets for larval stages [10]. Our results show that the caridina group achieved the highest final body weight, weight gain rate, and specific growth rate, significantly exceeding the tubifex and brine shrimp groups but not significantly different from the mealworm and raw fish block groups. These findings align with previous research identifying caridina and raw fish block as superior diets for juvenile salamanders. However, tubifex, commonly used in current production, did not demonstrate optimal growth performance, contrasting with Li et al.' s [17] conclusion that tubifex was the optimal feed among shrimp meat, fish meat, clam meat, and tubifex for juvenile sand goby (*Oxyeleotris marmoratus*). This discrepancy may relate to different feeding habits between species and the fact that tubifex and brine shrimp used in our experiment had higher moisture content, resulting in significantly lower dry matter intake per individual compared to other groups.

Animal weight gain is substantially influenced by dietary protein quality and amino acid composition; when dietary protein approximates ideal protein, animals achieve maximum daily weight gain and protein deposition [18]. Protein efficiency ratio reflects dietary protein quality, with tubifex showing the highest value and mealworm the lowest, indicating that tubifex protein is of high quality for juvenile salamanders, though lower total intake prevented optimal growth performance. Conversely, mealworms, despite high protein content, exhibited poor protein quality. The superior growth performance in the caridina and raw fish block groups may be attributed to significantly higher total protein and amino acid intake compared to other groups. However, the mealworm group, despite having the highest dry matter and protein intake, did not achieve optimal growth performance, possibly because excessive dietary protein and lipid levels are detrimental to fish growth and protein deposition [19], resulting in lower protein efficiency ratio. Additionally, during the experiment, salamanders in the mealworm group exhibited vigorous feeding behavior, with some individuals developing abdominal distension and floating, likely due to the high content of delicious amino acids (6.46%) in mealworms enhancing palatability. However, the incompletely developed digestive function of juvenile salamanders can lead to excessive food accumulation in the intestine, potentially causing mortality. The higher average weight in the mealworm group at day 20 may also be related to food accumulation in the intestine.

Although brine shrimp had higher nutritional content than tubifex, growth performance was lower in the brine shrimp group, which relates to the principle that feed size must match fish mouth gape [12]. During the first 20 days, salamander larvae showed weak size selectivity, resulting in similar average weights between brine shrimp and tubifex groups. However, as juveniles grew to 3-5 g, size selectivity increased, and the small size of brine shrimp reduced predation efficiency, resulting in the lowest dry matter intake and insufficient nutrition for growth. Zhang et al. [20] suggested that when energy intake cannot meet developmental requirements, larval fish growth becomes stunted. The lowest condition factor in the brine shrimp group indicates it is not an optimal feed for juvenile salamanders. Although the tubifex group did not achieve optimal growth performance, it exhibited the highest condition factor, possibly due to high protein efficiency ratio and essential amino acids to non-essential amino acids ratio. Yamamoto et al. [21] proposed that supplementation with crystalline essential amino acids can improve body composition and condition factor when dietary essential amino acids are deficient.

3.2 Effects of Different Diets on Whole-Body Conventional Nutritional Components and Amino Acid Composition

Fish quality is closely related to dietary nutritional composition. Protein content is a crucial indicator for evaluating nutritional value, while fat can improve meat flavor and tenderness; appropriate muscle fat content benefits taste, but excessive fat is detrimental to dietary health [22]. In this experiment, no significant differences in whole-body moisture or ash content were observed among groups. The caridina group showed the highest whole-body crude protein content, significantly exceeding the mealworm and brine shrimp groups but not significantly different from the raw fish block and tubifex groups. The high crude protein content in caridina and raw fish block resulted in good digestion and absorption, leading to higher whole-body crude protein content. Although mealworms contained the highest crude protein, whole-body crude protein was lower than in the caridina, raw fish block, and tubifex groups, possibly because excessive protein is detrimental to fish, increasing ammonia excretion and reducing protein efficiency [23], and potentially decreasing growth rate in some fish species [24]. Despite the lowest crude protein content in tubifex, whole-body crude protein in this group exceeded that of the mealworm and brine shrimp groups, possibly due to relatively high essential amino acid content. Chen et al. [25] demonstrated that supplementing low-protein diets with crystalline essential amino acids significantly increased whole-body crude protein in largemouth bass (*Micropterus salmoides*), though not to the level of fishmeal-based diets.

Whole-body crude fat content generally correlated positively with dietary crude fat content, with the mealworm group significantly higher than other groups, indicating that juvenile salamanders can absorb and utilize high-fat diets, potentially improving meat quality, though optimal lipid requirements require further investigation.

Wilson et al. [26] suggested that protein quality in formulated diets affects essential amino acid deposition rates in animal muscle, and that aquatic animal tissue essential amino acid composition is closely related to dietary amino acid content. Better-balanced and higher dietary essential amino acid content results in higher essential amino acid content in muscle tissue. Our results show that the essential amino acids to non-essential amino acids ratio in the raw fish block and mealworm groups was significantly lower than in the other three groups, differing from the ratio in the diets themselves, though different diets did not significantly affect individual amino acid content, total amino acids, or essential amino acids in whole-body composition. This suggests that dietary protein quality affects fish amino acid composition, with essential amino acid balance potentially being more important than total amino acid content. Limited research exists on amino acid requirements for Chinese giant salamander, but our results suggest that caridina amino acid composition may closely match juvenile salamander requirements.

3.3 Effects of Different Diets on Gastrointestinal Digestive Enzyme Activities

Fish digestive enzyme activity is closely related to the type and content of nutrients in the diet. Sun et al. [27] reported that protease activity in mandarin fish digestive tract increases with dietary crude protein content, with similar results in Jian carp [28] and rohu [29]. However, Li et al. [17] found that stomach protease activity in sand goby decreased with increasing dietary protein content, indicating that protease activity in aquatic animals is influenced not only by protein content but also by other factors. In our experiment, protease activity in the mealworm group was not highest, possibly due to excessive protein content or unbalanced amino acid composition. Su et al. [30] reported that at the same protein level, protease activity in yellow catfish (*Pseudobagrus fulvidraco*) fed mealworms was lower than in those fed fishmeal. Ali et al. [31] suggested that faster-growing fish have more active metabolism and higher protease activity, which aligns with our results showing higher protease activity in the faster-growing caridina and raw fish block groups and lower activity in the brine shrimp group.

Dietary lipid content induces lipase secretion, and lipase activity in yellow catfish larvae correlates positively with dietary fat content [32]. Fountoulaki et al. [33] found that increasing dietary fat content enhanced lipase activity in gilthead sea bream. Our experiment also revealed a positive correlation between gastrointestinal lipase activity and dietary crude fat content, as organisms adapt to feed characteristics to enhance nutrient absorption and transformation [34]. Compared to terrestrial animals, fish have lower demand and utilization capacity for carbohydrates like starch, particularly carnivorous fish with short digestive tracts [35]. Wang et al. [36] found that amylase activity in red sea bream larvae correlated positively with dietary starch content, with the highest activity in larvae fed formulated diets. Our results showed low gastrointestinal amylase activity in juvenile salamanders with minimal differences among groups, with only the brine shrimp group significantly lower. This may be related to the carnivorous nature of salamanders and low carbohydrate utilization, combined with inherently low starch content in all diets, resulting in non-significant differences except in the brine shrimp group. The low amylase activity in the brine shrimp group may be related to insufficient feed intake and malnutrition.

3.4 Feasibility Analysis of Different Diets in Production

Given the current challenge of expensive feed in Chinese giant salamander aquaculture, identifying optimal diets to reduce production costs is crucial. Diet selection should comprehensively consider nutritional value, particle size, availability, cost, and processing requirements. All five diets tested have applications in aquaculture. In terms of availability, tubifex and brine shrimp are widely available and inexpensive due to mature culture technologies, with market prices around 18 yuan/kg and even lower for bulk purchases. Mealworms are widely used in livestock and poultry production, with abundant market supply at approximately 14 yuan/kg, lower than tubifex and brine shrimp. Raw fish block sources are extensive, requiring only local purchase and processing of small trash fish, with variable regional prices; the fish block used in this experiment was farm-produced at 3-5 yuan/kg.

The caridina used in this experiment was naturally reproduced within the farm's water system at virtually no cost; large-scale supply requires only minimal fertilization and feeding of the water body. However, most salamander farms lack such water conditions for obtaining live caridina and must purchase frozen freshwater shrimp or marine shrimp from the market. Although supply is more limited than other diets, frozen marine shrimp are available at only 5-6 yuan/kg, with nutritional values potentially slightly different from our experimental caridina but likely having minimal impact.

Considering nutritional value and particle size, the caridina group achieved optimal growth performance, suggesting that caridina may most closely match juvenile salamander nutritional requirements. As farm-reproduced caridina can be selected for appropriate size, market-purchased products must be processed according to salamander size. Raw fish block ranked second in nutritional value but requires processing, especially for large-scale operations where processing small trash fish is labor-intensive. In such cases, slightly more expensive silver carp and bighead carp can be deboned and minced using a meat grinder for production application. Tubifex has slightly lower nutritional value than caridina and raw fish block but is appropriately sized for one-year-old juveniles without requiring processing, explaining its widespread current use. Mealworms have average nutritional value but excellent palatability; however, they are unsuitable for small juveniles due to risk of digestive issues and should only be used as a substitute when other feeds are insufficient. Brine shrimp have acceptable nutritional value but become unsuitable when salamanders exceed 3 g; they may be attempted during initial feeding stages.

Comprehensively evaluating multiple factors, caridina and raw fish block are feasible for production application and warrant promotion. While these diets may slightly reduce costs, the fundamental issue of high feed coefficients (4-5) for natural diets persists, keeping production costs high. Following the development pattern of feeds for species like sea bass, grouper, and Chinese sucker, formulated diets offer more comprehensive and balanced nutrition than natural diets, with adjustable nutritional levels and particle sizes tailored to fish requirements and lower costs. Therefore, developing formulated feeds is essential for the healthy and rapid development of Chinese giant salamander aquaculture.

In conclusion, under our experimental conditions and considering growth performance, body composition, and digestive enzyme activities comprehensively, caridina is the optimal diet for juvenile Chinese giant salamander, followed by raw fish block, with tubifex and mealworm being less suitable alternatives. Furthermore, the nutritional composition of caridina provides valuable reference for developing formulated feeds for juvenile Chinese giant salamander.

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