# Effects of different additives on growth, physiology and biochemistry and domestication rate of mandarin fish (*Siniperca chuatsi*)

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**Abstract**
This experiment aimed to study the acclimatization of mandarin fish fry, using squid paste, betaine, and black soldier fly larvae slurry as feeding agents to investigate their effects on the growth, physiological and biochemical characteristics, and acclimatization rate of mandarin fish larvae. The results showed that during the acclimatization process of mandarin fish fry, the weight gain rate of mandarin fish fed with black soldier fly larvae slurry and squid paste was higher than that of the control group; squid paste significantly affected the specific growth rate of mandarin fish. The feed coefficients for the three treatments were all lower than those of the control group, while the survival rate and acclimatization rate were both higher than those of the control group. In terms of physiological and biochemical indicators: the addition of three feed additives significantly increased the crude protein content in juvenile mandarin fish, while the addition of black soldier fly pulp significantly increased the crude fat content in the body. Squid paste significantly increased the activity of alanine transaminase and gamma-glutamyl transferase in the blood, and also significantly reduced the total protein content in the blood. Both squid paste and betaine significantly decreased the activity of alanine transaminase in the liver, but betaine also significantly reduced the activity of gamma-glutamyl transferase in the liver. Black soldier fly pulp and betaine both significantly increased the activity of glutamate dehydrogenase in the liver. In summary, all three feed additives can improve the efficiency of domestication. Among them, squid paste is most effective in promoting early growth performance and has excellent physiological metabolic indicators. Betaine, on the other hand, reduces the metabolic burden on the liver during the domestication process of mandarin fish. Therefore, it is worth considering the combination of squid paste and betaine for further validation.

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**Keywords:** Mandarin Fish, Additives, Growth, Domestication

## 1. Introduction

The mandarin fish (*Siniperca chuatsi*), also known as *osmanthus* fish or seasonal flower fish, belongs to the family *Siniperca* (Perciformes), subfamily Sinipercinae (Sinipercinae), and genus *Siniperca* (*Siniperca*). Its meat is tender and low in bones, rich in nutrients, and has a delicious taste, making it a unique and valuable freshwater fish in China, [1]. Currently, the development of mandarin fish feed farming has become a new trend in recent years. Raising mandarin fish using artificial feed not only significantly reduces breeding costs and improves efficiency but also provides an effective solution to the problem of mandarin fish diseases. However, there are challenges in acclimatizing fingerlings in feed farming, primarily due to the fact that artificial compound feed often leads to poor feeding behavior or refusal to eat. Additionally, feeding the feed in water for too long results in waste of feed and pollution of the aquaculture water body [2]. The low feeding rate of mandarin fish leads to insufficient energy intake, which directly affects the acclimatization rate and survival rate during the early seedling acclimatization stage. Therefore, adding feed attractants during this stage to promote feeding, improve acclimatization and survival rates, and reduce feed waste and pollution is particularly important [3]. This experiment focuses on mandarin fish seedlings, using squid paste, betaine, and black soldier fly larvae slurry as feed attractants to study their effects on the growth, physiological and biochemical characteristics, and acclimatization rate of mandarin fish fry. The aim is to evaluate the effectiveness of these feed attractants and provide theoretical support for early seedling acclimatization techniques and feed formulations in mandarin fish farming, with the hope of applying them in production practices and advancing the development of integrated compound feed farming and the mandarin fish industry.

## 2. Materials and methods

### 2.1. Experimental animals and feed

The fingerlings of Chinese perch, crucian carp, and silver carp feed fish are bred and hatched by Jingzhou Xinchuang Fishery Co., Ltd. During the juvenile fish acclimatization process, powdered feed and extruded feed are purchased from Guangdong Yuequn Biotechnology Co., Ltd., while the bait agent's squid paste and betaine are supplied by Jingzhou Zhanxiang Feed Co., Ltd. Black soldier fly larvae slurry is procured from Guangzhou Feixi Te Biotechnology Co., Ltd. When using powdered feed during acclimatization, the bait agent is added at 1.5% of the feed's weight. The bait agent is mixed with water and then blended with the powdered feed to form a paste for feeding.

### 2.2. Domestication of juvenile tuna feed

A total of 40,000 juvenile Chinese perch (*Siniperca chuatsi*) with an average body length of 4-6cm were randomly distributed into 8 net cages measuring 4×2×2m (2.5m depth) each, with 5,000 fish per cage. Each cage was equipped with an oxygenation system and internal micro-porous bottom aerators with recirculating water circulation. The study included 1 control group and 3 experimental groups, each with two replicates. The control group received no artificial feeders throughout the trial, while other procedures followed the experimental group. Experimental groups underwent sequential feeding regimens: live crucian carp fry + silver carp fry, semi-dead crucian fry + semi-dead silver carp fry, Chinese perch meal feed + dead crucian/fish fry, and Chinese perch meal feed (No.0). The first two days of the experimental group involved live crucian fry + silver carp fry, followed by semi-dead crucian/fish fry for 3 days. After 3 days of acclimatization, the group switched to Chinese perch No.0 feed mixed with dead crucian/fish fry. The feed was soaked in water before feeding, and the trial lasted 8 days. Three experimental groups received artificial feeders containing squid extract, betaine, and black soldier fly larvae slurry at 1.5% of feed weight. The feed was dissolved in water and thoroughly mixed with the feed. Feeding was conducted twice daily (5:30 AM and 6:30 PM) at fixed times, locations, and quantities. The feeding rate was maintained at 4%-5% daily until the fish were fully satiated. Before feeding, the micro-porous aerators were turned off while recirculating water was circulated to stimulate surface agitation, encouraging fish aggregation. Feeding was performed in small batches gradually. During the experiment, daily monitoring of water parameters included temperature, dissolved oxygen, pH, nitrite, and ammonia nitrogen levels. Feeding amounts were recorded each day. Post-experiment measurements covered mandarin fish body weight, weight gain rate, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate. Liver enzymes were analyzed through histological examination, while blood samples were drawn to measure serum enzyme activity levels. Whole fish specimens were preserved for comprehensive nutritional component testing.

### 2.3. Data processing

After the test, the average weight of each group of juvenile fish was measured. The calculation formula of growth index is as follows:

Weight gain rate WGR (%) = (average end weight-average initial weight)/average initial weight × 100%;

Specific growth rate SGR (%) /d = ln (average final weight/average initial weight) × 100% / number of experimental days;

Feed coefficient FCR =feed consumption/weight gain;

Survival rate SR (%) = (number of surviving animals / numbers of initial animals) × 100%;

Domestication rate DR (%) = (open feeding number/initial number) × 100%.

The data were statistically analyzed using SPSS 27.0. Before analysis, homogeneity of variance was tested. When the test was passed, one-way ANOVA was used with Duncan's method for multiple comparisons. Experimental results were expressed as mean ± standard error (Mean±S.E), with $P<0.05$ as the criterion for significant differences.

## 3. Results and analysis

### 3.1. Effects of different attractants on growth performance of juvenile Chinese perch

The growth performance of juvenile Chinese perch (*Siniperca chuatsi*) is shown in Table 1. The fish fed with black soldier fly larvae extract, squid meal, and betaine demonstrated higher weight gain rates than the control group, with significant differences observed in the groups supplemented with black soldier fly larvae extract and squid meal ($P<0.05$). Notably, the specific growth rate of fish fed squid meal showed a statistically significant advantage over the control group ($P<0.05$). All three experimental groups with added feed additives exhibited lower feed conversion ratios compared to the control group, while their survival and acclimatization rates remained higher than the baseline values.

**Table 1:** Effects of different attractants on growth performance of juvenile Chinese perch

| Parameter | Control | Black Soldier Fly Pulp | Squid Paste | Betaine |
|---|---|---|---|---|
| Initial Weight (IW, g) | 5.81 ± 0.14 | 5.81 ± 0.14 | 5.81 ± 0.14 | 5.81 ± 0.14 |
| Final Weight (FW, g) | 22.35 ± 1.21[a] | 24.12 ± 0.89[b] | 24.87 ± 0.78[b] | 23.45 ± 0.81[ab] |
| Weight Gain Rate (WG, %) | 284.68 ± 0.20[a] | 315.15 ± 0.19[b] | 328.06 ± 0.07[b] | 303.61 ± 0.31[ab] |
| Specific Growth Rate (SGR, %/day) | 16.84 ± 2.43[a] | 17.79 ± 1.04[ab] | 18.18 ± 1.37[b] | 17.44 ± 2.01[ab] |
| Feed Efficiency Ratio (FER) | 1.92 | 1.71 | 1.56 | 1.79 |
| Survival Rate (SR, %) | 71.2 | 79.3 | 81.5 | 75.6 |
| Domestication Rate (DR, %) | 77.5 | 85.2 | 85.8 | 84.0 |

**Note:** Different letters in the same row indicate highly significant differences (P<0.05), as shown in the following table.

### 3.2. Effects of different attractants on nutrient composition of juvenile mandarin fish

The addition of black soldier fly pulp, squid paste and betaine to the feed had no effect on the body moisture content of mandarin fish. However, the addition of the three bait agents significantly increased the crude protein content of juvenile mandarin fish (*P*<0.05), and the addition of black soldier fly pulp significantly increased the crude fat content of the body (*P*<0.05).

**Table 2:** Effects of different attractants on nutrient composition of juvenile mandarin fish

| Parameter | Control | Black Soldier Fly Pulp | Squid Paste | Betaine |
|---|---|---|---|---|
| Moisture Content (M, %) | 74.39 ± 0.81 | 73.89 ± 0.95 | 73.56 ± 0.41 | 72.56 ± 0.24 |
| Crude Protein (CP, %) | 18.63 ± 0.09[a] | 19.91 ± 0.11[b] | 19.65 ± 0.19[b] | 19.08 ± 0.18[a] |
| Crude Fat (CL, %) | 2.72 ± 0.22[a] | 3.87 ± 0.27[b] | 2.81 ± 0.15[a] | 2.87 ± 0.13[a] |

### 3.3. Effects of different attractants on serum biochemical indexes of juvenile Chinese perch

As shown in Table 3, the addition of squid extract and betaine to feed significantly increased serum aspartate aminotransferase (AST) activity (P<0.05), while squid extract also markedly elevated serum alanine aminotransferase (ALT) levels (P<0.05). The three feed additives showed no significant effects on alkaline phosphatase content (P>0.05). Notably, squid extract notably reduced total protein levels in blood samples (P<0.05).

**Table 3:** Effects of different attractants on serum biochemical indexes of juvenile Chinese perch

| Metric | Control | Black Soldier Fly Pulp | Squid Paste | Betaine |
|---|---|---|---|---|
| Glutamic-Oxalacetic Transaminase (AST) | 18.38 ± 3.73[a] | 20.13 ± 2.26[a] | 59.91 ± 5.78[c] | 48.64 ± 4.18[bc] |
| Glutamic-Pyruvic Transaminase (ALT) | 10.11 ± 1.79[a] | 11.23 ± 1.04[a] | 15.90 ± 2.24[b] | 13.77 ± 2.71[a] |
| Alkaline Phosphatase (AKP) | 48.33 ± 5.17 | 45.63 ± 3.59 | 50.78 ± 4.16 | 52.62 ± 4.56 |
| Total Protein (TP) | 26.46 ± 0.51[b] | 27.32 ± 1.23[b] | 23.14 ± 1.07[a] | 28.44 ± 1.36[b] |

### 3.4. Effects of different attractants on liver nitrogen metabolic enzyme activity in juvenile Chinese perch

As shown in Table 4, the addition of squid extract and betaine in the feed significantly reduced the activity of aspartate aminotransferase (AST) in the liver (*P*<0.05), while the addition of black soldier fly larvae extract showed no significant difference compared to the control group (*P*>0.05). Betaine supplementation significantly decreased the activity of alanine aminotransferase (ALT) in the liver (*P*<0.05), with no significant differences observed in other groups compared to the control group (*P*>0.05). Both black soldier fly larvae extract and betaine significantly increased the activity of glutamate dehydrogenase (GDH) in the liver (P<0.05), whereas squid extract showed no significant difference compared to the control group (*P*>0.05).

**Table 4:** Effects of different attractants on liver nitrogen metabolic enzyme activity in juvenile Chinese perch

| Metric | Control | Black Soldier Fly Pulp | Squid Paste | Betaine |
|---|---|---|---|---|
| Glutamic-Oxalacetic Transaminase (AST) | 59.46 ± 2.45[c] | 59.50 ± 1.38[c] | 40.35 ± 3.19[a] | 48.21 ± 1.65[b] |
| Glutamic-Pyruvic Transaminase (ALT) | 51.26 ± 3.50[b] | 43.76 ± 3.19[ab] | 46.61 ± 1.95[ab] | 37.56 ± 2.55[a] |
| Glutathione Dehydrogenase (GDH) | 217.08 ± 12.11[a] | 304.31 ± 17.26[bc] | 237.51 ± 18.97[ab] | 347.39 ± 20.55[c] |

## 4. Discussion

### 4.1. Effects of different attractants on growth performance of juvenile Chinese perch

This study evaluated three feed additives-black soldier fly larvae extract, squid extract, and betaine-during the full acclimatization period of 8 days for three experimental groups: feed fish, feed fish with powdered feed, and extruded granular feed. The squid extract group demonstrated remarkable growth performance: juvenile mandarin fish achieved a maximum weight gain of 328%, with a specific growth rate of 18.18% per day, survival rate of 81.5%, acclimatization rate of 85.8%, and feed conversion ratio (FCR) of 1.56. All growth parameters surpassed those of the other additives and control group. Results indicate that squid extract supplementation significantly enhances mandarin fish fry acclimatization success, promotes growth, and reduces feed conversion ratios. Wu Zunlin *et al*. established 44.7%-45.8% protein content as optimal for juvenile mandarin fish compound feed [4], achieving over 90% success within 5 days. Wu Fan *et al*. found that insect protein suspension feed improved meat quality, disease resistance, and survival rates in mandarin fish [5]. Aquatic animal feed additives enhance feed palatability by stimulating olfactory, gustatory, and visual cues, thereby increasing feeding motivation, accelerating consumption, and boosting intake [6]. Both squid extract and black soldier fly larvae extract proved effective as animal-derived feed enhancers. While betaine-a methyl-active substance—provides methyl donors with feed-stimulating properties [7], its effectiveness was less pronounced than animal-derived additives in this study, likely due to mandarin fish's unique taste receptors.

### 4.2. Effects of different attractants on nutrient composition of juvenile Chinese perch

Experimental findings indicate that the addition of black soldier fly larvae extract and squid meal significantly enhances crude protein content in mandarin fish fry during domestication. Squid meal, rich in free amino acids such as glycine, alanine, and glutamic acid with appetite-stimulating properties, improves feed palatability [8]. Previous studies have demonstrated that black soldier fly larvae extract enhances feed palatability in hybrid catfish diets, improves feed utilization efficiency, promotes growth, and increases crude protein deposition rate [9]. Fish growth is closely related to free amino acid content in their diet. Small peptides can boost the activity of specific digestive enzymes in fish, enabling more efficient nutrient absorption [10]. In this study, bioactive substances like free amino acids and small peptides from black soldier fly larvae extract and squid meal facilitated the balance and absorption of amino acids during mandarin fish growth, thereby improving feed efficiency and protein deposition. The addition of black soldier fly larvae extract also significantly increases crude fat content in fish bodies, which may be attributed to its lipid content.

### 4.3. Effects of different attractants on serum biochemical indexes of juvenile Chinese perch

ALT and AST are essential amino acid transfer enzymes widely present in animal liver cell mitochondria [11]. When the body is exposed to environmental stressors that damage cell membrane structures, these enzymes are released into the bloodstream, leading to elevated transaminase activity and disrupting normal metabolic processes in fish. In this study, the addition of squid extract and betaine significantly increased blood AST levels, while squid extract notably boosted ALT activity. This phenomenon may be attributed to its low freshness, as squid, being rich in moisture and unsaturated fatty acids, is prone to rancidity during storage, transportation, and feed processing [12]. Serum TP and ALB levels serve as critical indicators of protein synthesis metabolism [13]. The research revealed that dietary squid extract significantly reduced total blood protein content, which may relate to its freshness affecting gut immunity. However, its benefits in enhancing feed intake, improving weight gain, and optimizing feed utilization should not be overlooked. Therefore, fresh squid extract should be used during early acclimatization stages for short-term supplementation to avoid long-term adverse effects on fish immunity.

### 4.4. Effects of different attractants on the activity of liver nitrogen metabolism enzymes in juvenile Chinese perch

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver can reflect the degree of hepatocyte damage [14]. Appropriate supplementation of betaine in feed can reduce the AST and ALT activities in Oenirofe fish, thereby decreasing hepatic cell damage [15]. In this study, mandarin fish fed with betaine-based feed for 8 days exhibited lower ALT and AST levels in their livers, indicating reduced hepatocyte injury. This may be attributed to betaine's role as an efficient methyl donor that participates in choline intermediate metabolism, thereby reducing hepatic fat accumulation and hepatocyte damage [16]. Adding enzymatically hydrolyzed squid visceral extract to yellow catfish feed significantly reduces AST and ALT levels [17]. Our research also found that squid extract markedly decreases AST activity, which helps alleviate hepatic burden during mandarin fish acclimatization. Glutamate dehydrogenase (GDH), primarily located in the liver and acting as a key rate-limiting enzyme in amino acid deamination, reflects the metabolic processes of amino acids [18]. Results show that black soldier fly larvae extract and betaine significantly enhance hepatic GDH activity, indicating their influence on amino acid metabolism during mandarin fish acclimatization. When amino acid intake becomes imbalanced, catabolism outweighs anabolism, leading to energy consumption of stored amino acids [19]. This further confirms squid extract's growth-promoting effects during mandarin fish fry acclimatization.

## 5. Conclusions

The research findings demonstrate that all three feed additives can enhance acclimatization efficiency. Squid extract shows the most significant effects in promoting early growth performance with favorable physiological and metabolic indicators, while betaine effectively reduces hepatic metabolic burden during mandarin fish acclimatization. Therefore, combining squid extract and betaine may be a viable strategy worth exploring, though further validation is required.

## 6. Acknowledgement

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