Effects of Dietary Betaine Supplementation on Feeding, Growth Performance, Tissue Nutrient Composition, and Digestive Enzyme Activity in Hemifusus tuba (Postprint)

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Abstract

This study aimed to investigate the effects of dietary betaine supplementation on feeding, growth performance, tissue nutrient composition, and digestive enzyme activity in Hemifusus tuba. A total of 630 Hemifusus tuba with initial body weight of (22.54$±$0.25) g were randomly divided into 7 groups with 3 replicates per group and 30 individuals per replicate. The seven experimental groups were fed isonitrogenous and isolipidic experimental diets containing betaine at levels of 0 (control), 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2% for a period of 60 days. The results showed that when dietary betaine levels ranged from 0.2% to 0.8%, the feeding rate, weight gain rate, specific growth rate, and feed efficiency of Hemifusus tuba were significantly higher than those of the control group (P<0.05). The feeding rate, weight gain rate, and feed efficiency reached their maximum values at a betaine supplementation level of 0.6%, while the specific growth rate peaked at 0.4% supplementation. No significant differences were observed in survival rate among all groups (P>0.05). Dietary betaine levels had no significant effect on moisture and crude ash contents in muscle and hepatopancreas of Hemifusus tuba (P>0.05). The crude protein content in muscle was significantly higher in the 0.4%-1.2% groups compared with the control group (P<0.05), while crude fat content was significantly lower (P<0.05). In the hepatopancreas, the crude protein content was significantly higher in the 0.2%-1.2% groups compared with the control group (P<0.05), and crude fat content was significantly lower (P<0.05). With increasing dietary betaine levels, the activities of lipase, trypsin, and amylase in the hepatopancreas exhibited an initial increase followed by a decrease, reaching maximum values at a betaine level of 0.6%, which were all significantly higher than those of the control group (P<0.05). Based on these results, appropriate levels of dietary betaine promote feeding in Hemifusus tuba, increase crude protein content in muscle and hepatopancreas, decrease crude fat content in these tissues, and enhance the activities of lipase, trypsin, and amylase in the hepatopancreas, thereby benefiting the healthy growth of Hemifusus tuba. Using weight gain rate as the evaluation index, quadratic regression analysis indicated that the optimal dietary betaine supplementation level for Hemifusus tuba was 0.55%.

Full Text

Effects of Dietary Betaine on Feeding, Growth Performance, Tissue Nutritional Composition and Digestive Enzyme Activities of Hemifusus tuba Gmelin

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Abstract

This experiment was conducted to investigate the effects of dietary betaine supplementation on feeding, growth performance, tissue nutritional composition, and digestive enzyme activities of Hemifusus tuba Gmelin. A total of 630 snails with an initial body weight of (22.54$±$0.25) g were randomly divided into 7 groups, with 3 replicates per group and 30 snails per replicate. The seven experimental groups were fed isonitrogenous and isolipidic diets containing betaine at supplementation levels of 0 (control), 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2% for a 60-day trial period. The results showed that when dietary betaine levels ranged from 0.2% to 0.8%, the feeding rate, weight gain rate, specific growth rate, and feed efficiency of H. tuba were significantly higher than those of the control group (P<0.05). The feeding rate, weight gain rate, and feed efficiency reached their maximum values at a betaine supplementation level of 0.6%, while the specific growth rate peaked at 0.4% supplementation. No significant differences were observed in survival rates among all groups (P>0.05). Dietary betaine did not significantly affect moisture or ash content in muscle and hepatopancreas (P>0.05). However, crude protein content in muscle was significantly higher in the 0.4%-1.2% groups compared to the control (P<0.05), while crude lipid content was significantly lower (P<0.05). Similarly, crude protein content in hepatopancreas was significantly higher in the 0.2%-1.2% groups compared to the control (P<0.05), with crude lipid content significantly lower (P<0.05). As dietary betaine levels increased, the activities of lipase, trypsin, and amylase in hepatopancreas showed a trend of initially increasing and then decreasing. At a betaine supplementation level of 0.6%, the activities of lipase, trypsin, and amylase in hepatopancreas all reached their maximum values and were significantly higher than those of the control group (P<0.05). Based on these results, appropriate dietary betaine supplementation can promote feeding in H. tuba, increase crude protein content while decreasing crude lipid content in muscle and hepatopancreas, and enhance the activities of lipase, trypsin, and amylase in hepatopancreas, thereby supporting healthy growth. Using weight gain rate as the evaluation criterion, quadratic regression analysis indicated that the optimal dietary betaine supplementation level for H. tuba is 0.55%.

Key words: Hemifusus tuba Gmelin; betaine; feeding; growth performance; tissue nutritional composition; digestive enzyme

Betaine is a quaternary ammonium alkaloid widely found in animals, plants, and microorganisms, named after its isolation from sugar beet processing by-products [1]. It offers numerous advantages in aquaculture, including improving feed palatability, increasing feed intake, promoting animal growth, enhancing feed conversion efficiency, and reducing water pollution [2]. Additionally, betaine regulates water excretion from renal cells, improves sodium-potassium pump function, modulates osmotic pressure, maintains vitamin efficacy, alleviates stress, and functions as an antioxidant [3]. As a methyl donor, betaine participates in amino acid synthesis and synergistic effects, thereby playing a crucial role in protein synthesis and energy metabolism in tissues [4]. Currently, betaine is widely applied in aquaculture as a feed additive that demonstrates significant feeding attraction for many fish and shrimp species. For instance, appropriate dietary betaine supplementation has been shown to significantly improve weight gain rates in rainbow trout (*Oncorhynchus mykiss*) [5], Nile tilapia × blue tilapia hybrids (*Oreochromis niloticus* × *Oreochromis aureus*) [6], gibel carp (*Carassius auratus gibelio*) [7], giant freshwater prawn (*Macrobrachium rosenbergii*) [8], and whiteleg shrimp (*Penaeus vannamei*) [9]. However, the effects of betaine on feeding and growth in cultured mollusks require further investigation.

Hemifusus tuba Gmelin, commonly known as the trumpet whelk, inhabits soft mud and sandy bottoms at depths of 11-40 m in the sublittoral zone, primarily distributed along the coasts of Zhejiang Province and southward, including Guangdong, Guangxi, and Hainan in China [10]. Its soft body is large and plump, with tender meat and delicious flavor, rich in protein and unsaturated fatty acids, commanding high economic value and strong market demand both domestically and internationally [11]. Currently, all H. tuba sold in markets are wild-caught from coastal waters. However, due to overfishing and deteriorating marine environments in recent years, natural resources have become increasingly depleted, leading to supply shortages and rising prices, which creates promising prospects for aquaculture development. As a carnivorous species, H. tuba does not readily accept formulated feeds. Current culture practices primarily rely on bivalve prey such as clams (*Sinonovacula constricta*) and Manila clams (*Ruditapes philippinarum*), which increases production costs. These bivalves are also subject to seasonal availability and fishing moratoriums, making consistent supply challenging. Therefore, developing effective feeding attractants or feed additives for H. tuba formulated feeds is urgently needed. Previous research on H. tuba has mainly focused on intraspecific cannibalism [12], culture techniques [13-14], nutritional composition [15-16], and ecological habits [17-18], with limited reports on feed additives for formulated diets. Only one study by Zhou et al. [19] has investigated the effects of different feeding attractants on juvenile H. tuba. Consequently, this experiment employed a single-factor design to explore the effects of varying dietary betaine levels on feeding, growth performance, tissue nutritional composition, and digestive enzyme activities in H. tuba, providing a theoretical basis for developing formulated feeds for this species.

1.1 Experimental Design and Diet Preparation

Seven isonitrogenous and isolipidic experimental diets (crude protein 47%, crude lipid 7%) were formulated using domestic fish meal and Peruvian white fish meal as primary protein sources, α-starch as carbohydrate source, and mixed oil (fish oil:soybean oil = 1:1, Jinlongyu brand soybean oil) as lipid source. Betaine was supplemented at seven levels: 0 (control), 0.2%, 0.4%, 0.6%, 0.8%, 1.0%, and 1.2%. Dietary composition and nutrient levels are presented in Table 1 . Experimental betaine (purity $ $98%) and feed ingredients were purchased from Ningbo Tech-Bank Co., Ltd. Feed ingredients were ground and sieved through a 100-mesh screen. All ingredients and betaine were accurately weighed according to formulation ratios. Vitamin premix and mineral premix were incorporated using the progressive expansion method. Pre-cooked gelatin was added, followed by fish oil and soybean oil, and finally water. The mixture was blended uniformly using a feed mixer, formed into dough, and extruded through a manual meat grinder to produce pellets 2 mm in diameter and 10–15 mm in length. Pellets were dried at 60°C, stored in plastic bags, and kept at -20°C until use.

Table 1 Composition and nutrient levels of experimental diets (DM basis)

Item	Betaine supplemental level/%
Ingredients	
Dehulled soybean meal	
Dehulled peanut meal	
Domestic fish meal	
Peru white fish meal	
α-starch	
Vitamin premix[1]	
Mineral premix[2]	
Fish oil	
Soybean oil	
Dextrin	
Gelatin	
Total	
Additional betaine	
Nutrient levels[3]	
Crude protein	

Item	Betaine supplemental level/%
Crude lipid	
Ash	

[1]The vitamin premix provided the following per kg of diets: VA 2,500 IU, VD 500 IU, VE 70 mg, VK_3 25 mg, VB_1 16 mg, VB_2 10 mg, VB_6 18 mg, VB_{12} 0.05 mg, nicotinic acid 50 mg, D-pantothenic acid 45 mg, folic acid 3.5 mg, inositol 70 mg.

[2]The mineral premix provided the following per kg of diets: $FeSO_4 \cdot 7H_2O$ 6 mg, $CuSO_4 \cdot 5H_2O$ 1.5 mg, $MnSO_4 \cdot H_2O$ 16 mg, $ZnSO_4 \cdot 7H_2O$ 20 mg, $MgSO_4 \cdot 7H_2O$ 200 mg, $CaCl_2$ 65 mg, $CoCl_2$ 0.02 mg, KI 10 g, Na_2SeO_3 0.01 mg.

[3]Nutrient levels were all measured values.

1.2 Feeding Management

The feeding trial was conducted at Zhoujia Village Hatchery in Xiangshan County, Ningbo. Experimental snails were purchased from Shipu Fishing Port, Shipu Town, Xiangshan County, Zhejiang Province. Prior to the experiment, snails were acclimated in foam boxes (58.0 cm × 31.5 cm × 44.5 cm) for one week, fed the control diet until normal feeding behavior was observed, and then fasted for 24 hours before the formal trial began. A total of 630 snails with shell height (7.23$±0.25) $cm and body weight (22.54±0.25) g were randomly allocated into 7 groups, with 3 replicates per group and
$filtered, with a water depth of (15±1) cm, temperature (26.5±1.5)°C, salinity 22±$2, and pH 7.6–8.2. Continuous aeration was provided 24 h daily, maintaining dissolved oxygen concentration above 4.0 mg/L. The daily feeding rate was 6% of total body weight, with two feedings per day (06:00 and 17:00). Residual feed was collected 6 h after feeding, dried, and weighed. During the early culture period, water was exchanged every other day at 100% volume; during mid and late culture periods, water was exchanged daily at 50%–100% volume. The experimental period lasted 60 days.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection At the end of the feeding trial, after 24 h of fasting, snails were weighed and counted by replicate to determine feed intake and calculate feeding rate (FR), weight gain rate (WGR), specific growth rate (SGR), feed efficiency (FE), and survival rate (SR). Three snails were randomly selected from each replicate, and foot muscle and hepatopancreas were dissected, snap-frozen in liquid nitrogen, and stored at -80°C.

1.3.2 Determination of Nutritional Components in Muscle and Hepatopancreas Crude protein content was determined by the Kjeldahl method (GB/T 5009.5-2003), crude lipid by Soxhlet extraction (GB/T 5009.6-2003), moisture by drying method (GB/T 5009.3-2003), and ash by high-temperature incineration (GB/T 5009.4-2003).

1.3.3 Analysis of Digestive Enzyme Activities in Hepatopancreas Hepatopancreas tissues were minced and homogenized, and the supernatant was collected, aliquoted, and stored at -80°C until analysis. Activities of lipase, trypsin, and amylase were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.3.4 Calculation Formulas for Feeding and Growth Performance Indices Feeding rate [g/(d • individual)] = Σ(Wfr/N)/t
Weight gain rate (%) = 100 × (M_2 - M_1)/M_1
Specific growth rate (%/d) = 100 × (lnM_2 - lnM_1)/t
Feed efficiency (%) = 100 × (M_2 - M_1)/Mf
Survival rate (%) = 100 × N_2/N_1

Where: Wfr = total feed intake (g); N = number of snails per foam box; t = experimental duration (d); M_1 = initial body weight (g); M_2 = final body weight (g); Mf = dry matter intake (g); N_2 = number of surviving snails per box at trial end; N_1 = number of snails stocked per box at trial start.

1.4 Statistical Analysis

Experimental data are expressed as mean ± standard deviation. Data were processed using Excel 2003 and analyzed by one-way ANOVA using SPSS 19.0 software. Duncan' s multiple range test was used to compare differences among groups, with significance level set at $P < 0.05$.

2.1 Effects of Dietary Betaine on Feeding and Growth Performance of Hemifusus tuba Gmelin

As shown in Table 2 , feeding rate, weight gain rate, specific growth rate, and feed efficiency of H. tuba initially increased and then decreased with increasing dietary betaine levels. Feeding rate, weight gain rate, and feed efficiency reached maximum values at 0.6% betaine supplementation, while specific growth rate peaked at 0.4% supplementation. The 0.6% group showed no significant differences in weight gain rate, specific growth rate, or feed efficiency compared to the 0.4% group ($P > 0.05$), but these values were significantly higher than all other groups ($P < 0.05$). The feeding rate in the 0.6% group was significantly higher than all other groups ($P < 0.05$). At 1.2% betaine supplementation, weight gain rate and specific growth rate were significantly lower than the control group ($P < 0.05$), while feeding rate and feed efficiency showed no significant differences from the control ($P > 0.05$). No significant differences were observed in survival rates among all groups ($P > 0.05$).

Table 2 Effects of dietary betaine on feeding and growth performance of Hemifusus tuba Gmelin

Item	Betaine supplemental level/%
Feeding rate FR/[g/(d · individual)]	0.53$±0.02, $0.59±0.03^{bc}$, $0.63±0.01^{d}$, $0.66±0.01^{e}$, $0.62±0.03^{cd}$, $0.57±0.02^{b}$, $0.51±0.01$ ǀǀ*Weightga*

In the same row, values with different small letter superscripts indicate significant differences (P < 0.05), while values with the same or no superscripts indicate no significant differences (P > 0.05). The same applies below.

Regression equations were established using curve model fitting with feeding rate, weight gain rate, specific growth rate, and feed efficiency of H. tuba as dependent variables and dietary betaine level as the independent variable. The regression equation between feeding rate (y) and betaine level (x) was y = -0.339x^2 + 0.388x + 0.5296 (R^2 = 0.9644), indicating maximum feeding rate at 0.57% betaine supplementation (Figure 1 [Figure 1: see original paper]). The regression equation between weight gain rate (y) and betaine level (x) was y = -45.836x^2 + 50.748x + 43.617 (R^2 = 0.9584), indicating maximum weight gain rate at 0.55% betaine supplementation (Figure 2 [Figure 2: see original paper]). The regression equation between specific growth rate (y) and betaine level (x) was y = -0.4978x^2 + 0.5458x + 0.6067 (R^2 = 0.9706), indicating maximum specific growth rate at 0.55% betaine supplementation (Figure 3 [Figure 3: see original paper]). The regression equation between feed efficiency (y) and betaine level (x) was y = -14.372x^2 + 16.316x + 30.335 (R^2 = 0.9397), indicating maximum feed efficiency at 0.57% betaine supplementation (Figure 4 [Figure 4: see original paper]).

2.2 Effects of Dietary Betaine on Nutritional Components in Muscle and Hepatopancreas of Hemifusus tuba Gmelin

As shown in Table 3 , crude protein content in muscle initially increased and then decreased with increasing dietary betaine levels, reaching maximum value at 0.6% supplementation. The 0.2% group showed no significant difference from the control group (P > 0.05), but both were significantly lower than all other groups (P < 0.05). Crude lipid content in muscle initially decreased and then stabilized, with the 0.2% group showing no significant difference from the control (P > 0.05), but both were significantly higher than all other groups (P < 0.05). No significant differences were observed in moisture or ash content in muscle among all groups (P > 0.05). Crude protein content in hepatopancreas also showed an initial increase followed by a decrease with increasing betaine levels. No significant differences were detected among betaine-supplemented groups (P > 0.05), but all were significantly higher than the control (P < 0.05). Crude lipid content in hepatopancreas initially decreased and then increased, reaching minimum value at 0.8% betaine supplementation, with all betaine-supplemented groups significantly lower than the control (P < 0.05). No significant differences were observed in moisture or ash content in hepatopancreas among all groups ($P > 0.05$).

Table 3 Effects of dietary betaine on nutritional components in muscle and hepatopancreas of Hemifusus tuba Gmelin

<table>
<tr><th>Item</th><th>Betaine supplemental level/%</th></tr>
<tr><td>Muscle</td><td></td></tr>
<tr><td>Moisture</td><td>76.33$±0.76, 75.55±0.67, 75.78±1.04, 75.56±0.96, 75.23±1.03, 75.67±1.15, 75.93±1.22||Crudep
∗Hepatopancreas∗
∗|||Moisture|76.73±1.35, 76.54±2.01, 75.88±1.15, 76.49±1.13, 75.92±1.42, 76.64±0.98, 76.27±0</td></tr>
</table>

2.3 Effects of Dietary Betaine on Digestive Enzyme Activities in Hepatopancreas of Hemifusus tuba Gmelin

As shown in Table 4 , lipase, trypsin, and amylase activities in hepatopancreas initially increased and then decreased with increasing dietary betaine levels. Activities in the 0.2%-1.0% groups were significantly higher than those in the control and 1.2% groups ($P < 0.05$). At 0.6% betaine supplementation, lipase, trypsin, and amylase activities in hepatopancreas reached their highest values. Except for lipase and amylase activities, which showed no significant differences from the 0.4% and 0.8% groups ($P > 0.05$), all three enzyme activities were significantly higher than those in all other groups ($P < 0.05$).

Table 4 Effects of dietary betaine on digestive enzyme activities in hepatopancreas of Hemifusus tuba Gmelin (U/g prot)

<table>
<tr><th>Item</th><th>Betaine supplemental level/(%)</th></tr>
<tr><td>Lipase</td><td>29.12$±1.25, 32.71±2.18^b, 36.65±1.77^c, 37.29±1.91^c, 35.18±0.94^{bc}, 33.37±2.32^b, 28.52±0.63||Pr</td></tr>
</table>

3.1 Effects of Dietary Betaine on Feeding and Growth Performance of Hemifusus tuba Gmelin

Betaine is a quaternary ammonium alkaloid with a chemical structure similar to glycine, producing a slightly bitter sweet taste that creates a unique flavor preferred by fish, crustaceans, and other aquatic animals. This stimulates the olfactory and gustatory senses, enhances secretion of digestive glands, and substantially increases feed intake [20]. Additionally, betaine can mask undesirable flavors in formulated feeds, promoting consumption of otherwise unpalatable nutrients, reducing feed waste, and improving feed conversion efficiency, making it a highly effective chemical feeding attractant in aquaculture [21]. Studies have shown that betaine increases secretion of triiodothyronine (T3) and insulin in aquatic animals, regulating metabolic activities through neuroendocrine pathways to achieve growth-promoting effects [22]. Similar to results reported in cobia (*Rachycentron canadum*) [23], rohu (*Labeo rohita*) [24], and chinook salmon

(*Oncorhynchus tshawytscha*) [25], our findings demonstrate that betaine exhibits strong feeding attraction for H. tuba. Within the range of 0–0.6% supplementation, increasing betaine levels progressively enhanced feeding rate, weight gain rate, specific growth rate, and feed efficiency, with significant differences from the control group, indicating stronger olfactory and gustatory stimulation and greater feed intake at higher supplementation levels within this range.

Methyl donors generally cannot be synthesized in animal bodies and must be obtained from dietary sources. Methyl groups are essential for various metabolic processes and play vital roles in the nervous, endocrine, immune, urinary, and cardiovascular systems. Insufficient or excessive methyl donors can cause growth disorders and pathological conditions [26]. Betaine serves as an efficient methyl donor, providing active methyl groups that spare amino acids for protein synthesis [27]. However, research indicates that when dietary betaine exceeds optimal levels, growth-promoting effects decline rapidly in species such as rice field eel (*Monopterus albus*) [28] and tambaqui (*Colossoma brachypomum*) [29]. In our study, feeding rate and weight gain rate began to decrease when betaine supplementation exceeded 0.6%, suggesting this level satisfies growth, nutritional, and metabolic requirements for H. tuba. Notably, at 1.2% supplementation, both feeding rate and weight gain rate were lower than the unsupplemented control, indicating that excessive betaine inhibited growth.

Beyond its feeding attraction properties, betaine can enhance immune capacity, prevent diseases, and improve survival in some aquatic animals. For example, dietary betaine significantly increased survival rates in pike perch (*Sander lucioperca*) [30] and rice field eel [28]. In contrast, our study found no significant effect of betaine on H. tuba survival, consistent with findings in kutum (*Rutilus frisii kutum*) [31] and blue tilapia (*Oreochromis aureus*) [32], suggesting that betaine' s effects on survival vary among species.

3.2 Effects of Dietary Betaine on Tissue Nutritional Composition of Hemifusus tuba Gmelin

In addition to promoting growth, betaine improves nutritional composition and flesh quality in aquatic animals. For instance, appropriate betaine supplementation significantly increased crude protein content in muscle of Caspian roach (*Rutilus rutilus caspicus*) [33], while crude lipid content in muscle and liver of rice field eel decreased with increasing betaine levels [34]. Dietary betaine at 0.01% significantly reduced crude lipid content in muscle and liver of gibel carp, and at 0.10% supplementation decreased serum triglyceride and total cholesterol by 7.23% and 15.86%, respectively [35]. In our study, betaine supplementation did not significantly affect moisture or ash content in muscle or hepatopancreas of H. tuba, but moderately increased crude protein content while decreasing crude lipid content. These effects occur because: (1) betaine synthesizes phosphatidylcholine to participate in lipid transport and provides methyl groups to substitute for methionine in lipid metabolism, thereby sparing methionine for protein synthesis; and (2) betaine increases homocysteine-S-methyltransferase activity, promoting conversion of homocysteine to methionine, an essential amino acid primarily used for protein synthesis, thus achieving a net increase in methionine availability [26]. Furthermore, betaine accelerates fatty acid oxidation in mitochondrial cells, increases long-chain acylcarnitine content and the ratio of long-chain acylcarnitine to free carnitine in muscle and hepatopancreas, promotes lipid decomposition, reduces fat deposition, and effectively prevents fatty liver disease [22,36]. Therefore, betaine supplementation promotes protein synthesis and deposition while enhancing lipid catabolism.

3.3 Effects of Dietary Betaine on Digestive Enzyme Activities in Hepatopancreas of Hemifusus tuba Gmelin

Digestive enzyme activity is a critical indicator of digestive physiology and nutrient utilization capacity, directly affecting nutrient absorption and utilization [37]. When aquatic organisms exhibit rapid growth, their metabolism is more active and digestive enzyme activities are relatively higher. Betaine enhances digestive and absorptive functions of hepatopancreas through feeding attraction and participation in methylation processes. Yan et al. [38] found that protease and amylase activities in hepatopancreas of Nile tilapia (*Tilapia nilotica*) peaked at dietary betaine levels of 1.5% and 0.8%, respectively. Research showed maximum protease activity in hepatopancreas of longsnout catfish (*Leiocassis longirostris*) at 0.7% betaine supplementation [39], significantly increased intestinal amylase and protease activities in channel catfish (*Ictalurus punctatus*) at 0.5% supplementation [40], and maximum activities of protease, trypsin, amylase, and lipase in stomach, intestine, and hepatopancreas of rice field eel at 1.5% supplementation [41]. In our study, lipase, trypsin, and amylase activities in H. tuba hepatopancreas were highest at 0.6% betaine supplementation. This suggests that betaine-induced feeding stimulates hepatopancreas to secrete more enzymes related to lipid, starch, and protein digestion, facilitating better nutrient utilization from feed and promoting growth. Additionally, we observed higher amylase activity than lipase activity in H. tuba hepatopancreas, similar to findings in the spider crab (*Hyas araneus*) [42].

4 Conclusion

Appropriate dietary betaine supplementation can promote feeding in Hemifusus tuba Gmelin, increase crude protein content while decreasing crude lipid content in muscle and hepatopancreas, and enhance activities of lipase, trypsin, and amylase in hepatopancreas, thereby supporting healthy growth. Using weight gain rate as the evaluation criterion, quadratic regression analysis indicates that the optimal dietary betaine supplementation level for H. tuba is 0.55%.

References

[1] Feng Y, Xu HJ, Liu D, et al. Application of betaine in aquatic animal production [J]. Feed Review, 2013(8): 38-40.

[2] TIRIL S U, ALAGIL F, YAGCI F B, et al. Effects of betaine supplementation in plant protein based diets on feed intake and growth performance in rainbow trout (*Oncorhynchus mykiss*) [J]. The Israeli Journal of Aquaculture, 2008, 60(1): 57-64.

[3] Gao YA, Xia RL, Zeng HS, et al. Effect of dietary betaine on growth of *Silurus meridionalis* [J]. Inland Fisheries, 2001(10): 7.

[4] Hao SY, Liu LS, Wang GD, et al. Effects of dietary betaine on performance, egg quality and serum biochemical indices of laying hens under heat stress [J]. Chinese Journal of Animal Nutrition, 2017, 29(1): 184-192.

[5] BEKLEVIK G, POLAT A. Effects of DL-alanine and betaine supplemented diets on the growth composition fingerling rainbow trout (*Oncorhynchus mykiss*, W. 1972) [J]. Turkish Journal of Veterinary and Animal Sciences, 2001, 25(3): 301-307.

[6] Zhu DG, Yu D, Chen T, et al. Effects of betaine on growth, body lipid content and serum biochemical indices of Nile tilapia × blue tilapia hybrids [J]. Journal of Shanghai Ocean University, 2011, 20(2): 224-229.

[7] Huan HL, Wang YF, Zhou WR, et al. Effects of L-carnitine, betaine and choline chloride on growth performance and muscle quality of gibel carp [J]. Feed Industry, 2009, 30(24): 31-33.

[8] FELIX N, SUDHARSAN M. Effect of glycine betaine, a feed attractant affecting growth and feed conversion of juvenile freshwater prawn *Macrobrachium rosenbergii* [J]. Aquaculture Nutrition, 2004, 10(3): 193-197.

[9] PONPAI T, PENPRAPAI N, LERSSUTTHICHAWAL T, et al. Effect of betaine on growth performance resistance Vibriosis white shrimp (*Penaeus vannamei*) [J]. Songklanakarin Journal of Science and Technology, 2007, 29(5): 1275-1282.

[10] Yang ZQ, Li RH, Mu CK, et al. Fluorescence microscopic observation of fertilization process and analysis of nurse egg formation in *Hemifusus tuba* [J]. Journal of Fisheries of China, 2016, 40(11): 1674-1682.

[11] Wang WJ, Pan YD, Zhou YN, et al. Histological and anatomical study and scanning electron microscopy observation of sexual abnormality in *Hemifusus tuba* [J]. Journal of Fisheries of China, 2014, 38(11): 1865-1878.

[12] Mortem B. Juvenile growth of the South China Sea whelk *Hemifusus tuba* (Gmelin) (Prosobranchia: Melongenidae) and the importance of sibling cannibalism in estimates of consumption [J]. Journal of Experimental Marine Biology and Ecology, 1987, 109(1): 1-14.

[13] Wang BB, Wang MF, Li SB, et al. Preliminary study on cage hanging culture techniques for juvenile *Hemifusus tuba* [J]. Journal of Tropical Biology, 2012, 3(1): 83-87.

[14] Luo J, Liu H, Li F, et al. Comparison of *Hemifusus tuba* culture in tidal flat net enclosures, cages and cement ponds [J]. Journal of Aquaculture, 2016, 37(12): 1-6.

[15] Zhu AY, Zhao XJ, Yang YQ. Analysis of nutritional components in soft body of *Hemifusus tuba* from Dongji sea area [J]. South China Fisheries Science, 2008, 4(2): 63-68.

[16] Jiang XM, Jiang XM, Du XX, et al. Analysis of nutritional components in muscle of different sizes of *Hemifusus tuba* [J]. Chinese Journal of Animal Nutrition, 2012, 24(10): 2059-2066.

[17] Luo J, Liu CW, Huang XH. Effects of salinity on embryonic development of *Hemifusus tuba* [J]. Journal of Guangdong Ocean University, 2007, 27(3): 24-28.

[18] Du T, Luo J, Liu CW, et al. Effects of temperature on embryonic development of *Hemifusus tuba* Gmelin [J]. Marine Sciences, 2010, 34(6): 44-49.

[19] Zhou SN, Du XX, Peng RB, et al. Effects of different feeding attractants on feeding and growth of juvenile *Hemifusus tuba* [J]. Journal of Ningbo University (Natural Science & Engineering Edition), 2017, 30(6): 42-47.

[20] Yan XZ. Function and mechanism of betaine as fish feed additive [J]. Feed Research, 1997(7): 2-4.

[21] Gong HW, Chen K. Preliminary study on attractive effect of betaine solution on behavioral orientation of *Pelteobagrus fulvidraco* [J]. Freshwater Fisheries, 2003, 33(2): 13-15.

[22] Lu QE, Li XX, Li ZQ. Mechanism of betaine promoting growth of *Colossoma brachypomum* [J]. Journal of Fisheries of China, 2003, 27(6): 564-569.

[23] Zhang WC, Liang GY, Yang HJ, et al. Effects of dietary betaine on growth, body composition and serum biochemical indices of cobia (*Rachycentron canadum*) [J]. South China Fisheries Science, 2012, 8(3): 1-9.

[24] SHANKAR R, MURTHY H S, PAVADI P, et al. Effect of betaine as a feed attractant on growth, survival, and feed utilization in fingerlings of the Indian major carp, *Labeo rohita* [J]. The Israeli Journal of Aquaculture-Bamidgeh, 2008, 60(2): 95-99.

[25] CLARKE W C, VIRTANEN E, BLACKBURN J, et al. Effects of a dietary betaine/amino acid additive on growth and seawater adaptation in yearling chinook salmon [J]. Aquaculture, 1994, 121(1/2/3): 137-145.

[26] Zhan G. Effects of dietary L-carnitine and betaine on growth and lipid metabolism of tilapia [D]. Master' s Thesis. Nanning: Guangxi University, 2008.

[27] Huan HL, Wang YF, Zhou WR, et al. Effects of different alkaloids on growth and lipid metabolism of gibel carp [J]. Jiangsu Agricultural Sciences, 2010(6): 375-377.

[28] Feng JB, Ruan GL, Yang DQ. Effects of different betaine doses on survival, growth and immunity of rice field eel [J]. Feed Industry, 2006, 27(14): 27-28.

[29] Lu QE, Li ZQ, Li XX. Effects of betaine hydrochloride on lipid metabolism of *Colossoma brachypomum* [J]. Oceanologia et Limnologia Sinica, 2003, 34(3): 306-312.

[30] ZAKIPOUR R E, AKBARI M, ARSHADI A, et al. Effect of different levels of dietary betaine on growth performance, food efficiency and survival rate of pike perch (*Sander lucioperca*) fingerlings [J]. Iranian Journal of Fisheries Sciences, 2012, 11(4): 902-910.

[31] JABARI E, AKRAMI R, CHITSAZ H, et al. Effect of betaine as a feed attractant on growth, survival, body composition and response to environmental stress in Kutum (*Rutilus frisii kutum*) fingerling [J]. Iranian Scientific Fisheries Journal, 2017, 26(1): 83-93.

[32] GENC M A, TEKELIOGLU N, YILMAZ E, et al. Effect of dietary betaine on growth performance and body composition of *Oreochromis aureus* reared in fresh and sea water: A comparative study [J]. Journal of Animal and Veterinary Advances, 2006, 5(12): 1185-1188.

[33] FATTAHI S, HOSSEINI S A, SUDAGAR M. The effects of dietary betaine on growth performance and carcass synthesis in Caspian roach (*Rutilus rutilus caspicus*) fingerlings [J]. Scientific Journal of Animal Science, 2013, 2(5): 132-137.

[34] Chen F, Yang DQ, Ruan GL, et al. Effects of betaine on growth and lipid content in muscle and liver of rice field eel [J]. Journal of Hubei Agricultural College, 2004, 24(3): 190-192.

[35] Li HX, Liu WB, Li XF, et al. Effects of dietary choline chloride, betaine and lysophospholipid on growth, lipid metabolism and hematological indices of gibel carp [J]. Journal of Fisheries of China, 2010, 34(2): 292-299.

[36] Luo L, Gao P, Xue M, et al. Effects of compound betaine on growth and lipid metabolism of common carp [J]. Feed Research, 2003(8): 1-3.

[37] Jin LB, Zhang LL, Li RH, et al. Effects of different diets on growth, hepatopancreas digestive enzyme activities and soft body nutritional composition of *Hemifusus tuba* [J]. Marine Sciences, 2013, 37(6): 66-72.

[38] Yan XZ, Qiu LQ. Effects of dietary betaine on protease and amylase activities in hepatopancreas of Nile tilapia [J]. Journal of Fishery Sciences of China, 1997, 4(1): 88-93.

[39] Yang JQ, Huang J. Effects of betaine on protease activities in major digestive organs of longsnout catfish [J]. Feed Industry, 2006, 27(22): 22-24.

[40] Wu L, Chen PF, Luo SL, et al. Effects of different additives on intestinal protease and amylase activities of channel catfish [J]. Feed Research, 2002(1): 4-7.

[41] Hu WB, Ruan GL, Yang DQ. Effects of betaine on digestive enzyme activities of rice field eel [J]. Journal of Yangtze University (Natural Science Edition), 2011, 8(3): 229-232.

[42] HIRCHE H J, ANGER K. Digestive enzyme activities during larval development of *Hyas araneus* (Decapoda, Majidae) [J]. Comparative Biochemistry and Physiology B: Comparative Biochemistry, 1987, 87(2): 297-302.

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