

Effects of Dietary Taurine Levels on Growth Performance and Body Composition of Juvenile *Epinephelus coioides* at Different Growth Stages: Postprint

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Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary taurine levels on growth performance and body composition of juvenile orange-spotted grouper (*Epinephelus coioides*) at different growth stages. Using casein and gelatin as protein sources, experimental diets with taurine levels of 0 (D1), 0.5% (D2), 1.0% (D3), and 1.5% (D4) were formulated. Juvenile orange-spotted grouper with initial body weight of (13.85 ± 0.25) g were randomly divided into 4 groups, with 4 aquaria (volume 120 L) per group, and 25 fish per aquarium. The experimental fish were fed the diets twice daily to apparent satiation for a culture period of 84 d, and were weighed and sampled on days 28, 56, and 84 of feeding. Days 1-28 of the experiment constituted the first growth stage, days 29-56 the second growth stage, and days 57-84 the third growth stage. The results showed that at each growth stage, the weight gain rate, specific growth rate, feeding rate, and feed efficiency of fish in the taurine-supplemented groups (D2, D3, and D4) were significantly higher than those in the non-taurine-supplemented group (D1) ($P < 0.05$), and the weight gain rate of fish in group D3 was significantly higher than that in the other taurine-supplemented groups (except for group D4 in the third growth stage) ($P < 0.05$). At the same dietary taurine level, the weight gain rate, specific growth rate, feeding rate, and feed efficiency of fish in the first growth stage were significantly higher than those in the second and third growth stages ($P < 0.05$). On days 28, 56, and 84 of feeding, the hepatosomatic index and viscerosomatic index of fish in all taurine-supplemented groups were significantly lower than those in the non-taurine-supplemented group ($P < 0.05$), while the condition factor showed no significant difference from the non-taurine-supplemented group ($P > 0.05$). At the same dietary taurine level, the hepatosomatic index and viscerosomatic index on days 56 and 84 were significantly lower than those on day 28 ($P < 0.05$). On day 84 of feeding, the crude protein content

of fish body in all taurine-supplemented groups was significantly higher, while the crude lipid content was significantly lower, than those in the non-aurine-supplemented group ($P < 0.05$). These results indicate that maintaining a certain level of dietary taurine is beneficial for the growth of juvenile orange-spotted grouper, while both deficiency and excess of dietary taurine are detrimental to its growth. Dietary taurine supplementation reduced body lipid deposition and increased body protein deposition in juvenile orange-spotted grouper. Using weight gain rate as the evaluation index, regression analysis yielded optimal dietary taurine levels for juvenile orange-spotted grouper of 1.20% for the first growth stage (body weight: 13.83–39.09 g), 1.08% for the second growth stage (body weight: 39.09–66.88 g), and 1.00% for the third growth stage (body weight: 66.88–101.03 g). The taurine requirement of juvenile orange-spotted grouper decreased with increasing fish age.

Full Text

Effects of Dietary Taurine Level on Growth Performance and Body Composition of Juvenile Grouper (*Epinephelus coioides*) at Different Growth Periods

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Abstract

This study investigated the effects of dietary taurine level on growth performance and body composition of juvenile grouper (*Epinephelus coioides*) at different growth periods. Four experimental diets were formulated using casein and gelatin as protein sources to contain 0 (D1), 0.5% (D2), 1.0% (D3), and 1.5% (D4) taurine. Juvenile grouper with initial body weight of (13.85 ± 0.25) g were randomly divided into 4 groups, with 4 aquariums per group (120 L each) at a stocking density of 25 fish per aquarium. The 84-day feeding trial was divided into three growth periods: days 1–28 (1st period), days 29–56 (2nd period), and days 57–84 (3rd period). Fish were weighed and sampled on days 28, 56, and 84. Results showed that at each growth period, weight gain ratio (WGR), specific growth rate (SGR), feeding rate (FR), and feed efficiency (FE) in taurine-supplemented groups (D2, D3, D4) were significantly higher than those in the non-aurine group (D1) ($P < 0.05$). The D3 group exhibited significantly higher WGR than other taurine groups (except D4 at the 3rd period) ($P < 0.05$). At the same dietary taurine level, WGR, SGR, FR, and FE at the 1st growth period were significantly higher than those at the 2nd and 3rd periods ($P < 0.05$). On days 28, 56, and 84, hepatosomatic index (HSI) and viscerasomatic index (VSI) in taurine groups were significantly lower than in the

non-aurine group ($P < 0.05$), while condition factor (CF) showed no significant difference ($P > 0.05$). At the same dietary taurine level, HSI and VSI on days 56 and 84 were significantly lower than on day 28 ($P < 0.05$). On day 84, whole-body crude protein content was significantly higher while crude lipid content was significantly lower in taurine groups compared to the non-aurine group ($P < 0.05$). These results indicate that appropriate dietary taurine level benefits growth of juvenile grouper, while deficiency or excess is detrimental. Dietary taurine supplementation reduces body lipid deposition but increases protein deposition. Based on WGR as the evaluation index, regression analysis revealed optimal dietary taurine levels of 1.20% (1st period, body weight: 13.83–39.09 g), 1.08% (2nd period, body weight: 39.09–66.88 g), and 1.00% (3rd period, body weight: 66.88–101.03 g), demonstrating that taurine requirement decreases with fish age.

Key words: juvenile grouper (*Epinephelus coioides*); dietary taurine level; growth period; growth performance; body composition

Introduction

Taurine is the most abundant free amino acid that does not participate in protein metabolism in animals and plays multiple important biological roles in regulating physiological functions [1]. A key function of taurine is its conjugation with bile acids in the liver to form bile salts, which enhances the activity of the rate-limiting enzyme cholesterol 7-hydroxylase, promotes cholesterol conversion to bile acids, and facilitates lipid digestion and metabolism [2]. At fixed dietary protein and lipid levels, taurine supplementation can increase whole-body crude protein and lipid content in grass carp (*Ctenopharyngodon idella*) [3], but decrease crude lipid content in rainbow trout [4] (*Oncorhynchus mykiss*), Atlantic salmon [5] (*Salmo salar*), and turbot [6] (*Scophthalmus maximus*). Our previous studies also found that dietary taurine increased crude lipid content in Nile tilapia [7] (*Oreochromis niloticus*) but decreased it in grouper [8] (*Epinephelus coioides*), indicating a close relationship between taurine and lipid metabolism in fish. Taurine is also a conditionally essential amino acid. Except for a few mammals such as felids that cannot synthesize taurine, most vertebrates possess some taurine synthesis capacity; however, during early life stages, endogenous synthesis is relatively insufficient to meet growth and development requirements, necessitating exogenous supplementation [9]. In fish, dietary taurine supplementation improves growth performance in turbot [2] and Japanese flounder [10] (*Paralichthys olivaceus*), while taurine deficiency inhibits growth in cobia [11] (*Rachycentron canadum*) and rainbow trout [12], and may even cause green liver syndrome [13–14].

Fish require different taurine levels at different growth stages. For example, taurine requirement in Japanese flounder larvae (body weight: 2.4 g) is three times that of juveniles (body weight: 14.5 g) [15]; however, no studies have

reported taurine requirements during different juvenile stages. The juvenile period is a critical transition from larval to mature stage, characterized by rapid organ and tissue development, with corresponding changes in nutrient requirements. Therefore, this study formulated isonitrogenous and isolipidic diets with different taurine levels and established three growth periods (each 28 days) to conduct an 84-day feeding trial on juvenile grouper, aiming to explore the relationship between dietary taurine level and growth at different juvenile stages through growth performance and body composition analysis, thereby providing a basis for taurine application in aquafeeds.

Materials and Methods

1.1 Experimental Diets Casein and gelatin (taurine-free) served as protein sources, fish oil, soybean oil, and soybean lecithin as lipid sources, corn starch as carbohydrate source, and microcrystalline cellulose as filler. Four experimental diets were formulated to contain 0 (D1), 0.5% (D2), 1.0% (D3), and 1.5% (D4) taurine. Casein, gelatin, and shrimp meal were ground to pass through an 80-mesh sieve, then all ingredients were mixed with water according to dietary formulas and pelleted into 2.5 mm diameter pellets.

1.2 Culture Management After two weeks of acclimation, 400 healthy juvenile grouper of uniform size were randomly stocked into 16 aquariums (120 L each) at 25 fish per aquarium. The aquariums were randomly divided into 4 groups with 4 replicates per group. Initial body weight was (13.85 ± 0.25) g. Fish were hand-fed to apparent satiation twice daily at 08:30 and 18:30. Uneaten feed and feces were removed 0.5 h after feeding, and daily water exchange was approximately 100%. Fish behavior and feeding were observed and recorded daily throughout the 84-day culture period: days 1-28 constituted the 1st growth period, days 29-56 the 2nd period, and days 57-84 the 3rd period. Water temperature was maintained at 24.5-30.8 °C, and dissolved oxygen concentration was >5.7 mg/L.

1.3 Sample Collection At the start of the trial and on days 28, 56, and 84, all fish in each aquarium were removed, bulk-weighed, and returned to their respective aquariums after 24 h stabilization. Six fish per aquarium were then randomly selected, anesthetized with eugenol, and individually measured for body weight and length. Livers and other viscera were dissected and weighed. On day 84, three fish per aquarium were randomly sampled and stored at -20 °C for whole-body composition analysis.

1.4 Analytical Methods

1.4.1 Proximate Composition Analysis Moisture content in feed ingredients, experimental diets, and fish samples was determined by oven drying at 105 °C to constant weight. Crude protein content was measured by the Kjeldahl

method using a Kjeltex 8400 analyzer. Crude lipid content was determined by Soxhlet extraction, and crude ash content by muffle furnace incineration at 550 °C.

1.4.2 Taurine Content Determination Dietary taurine was extracted following the method of Zhou et al. [7] and analyzed using a Hitachi L-8900 amino acid analyzer.

1.5 Growth Performance Calculations Growth parameters were calculated as follows:

$$\text{Weight gain ratio (WGR, \%)} = 100 \times (\text{Wt} - \text{W0}) / \text{W0}$$

$$\text{Specific growth rate (SGR, \% / d)} = 100 \times (\ln \text{Wt} - \ln \text{W0}) / t$$

$$\text{Feeding rate (FR, \% / d)} = 100 \times \text{Wf} / [(\text{Wt} / 2 + \text{W0} / 2) / t]$$

$$\text{Feed efficiency (FE)} = (\text{Wt} - \text{W0}) / \text{Wf}$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times \text{Wh} / \text{Wb}$$

$$\text{Viscerasomatic index (VSI, \%)} = 100 \times \text{Wv} / \text{Wb}$$

$$\text{Condition factor (CF, g / cm}^3\text{)} = 100 \times \text{Wb} / \text{L}^3$$

Where W0 = initial mean weight (g), Wt = final mean weight (g), Wf = total feed intake (g), Wb = sample fish body weight (g), Wh = sample fish liver weight (g), Wv = sample fish viscera weight (g), L = sample fish body length (cm), and t = feeding days (d).

1.6 Statistical Analysis Data were analyzed by one-way and two-way ANOVA using SPSS 17.0. All data are presented as mean \pm SD. When significant differences were detected, multiple comparisons were performed using Student-Newman-Keuls test with significance set at $P < 0.05$.

Results

2.1 Effects of Dietary Taurine Level on Growth Performance Results are presented in and . At each growth period, WGR, FE, SGR, and FR in all taurine-supplemented groups (D2, D3, D4) were significantly higher than in the non-taurine group (D1) ($P < 0.05$). WGR increased initially then decreased with increasing dietary taurine level; D3 group showed significantly higher WGR than other taurine groups at the same period, except for D4 at the 3rd period ($P < 0.05$). Except for significantly higher SGR in D3 than D4 at the 2nd period ($P < 0.05$), no significant differences were observed in SGR, FE, and FR among taurine groups at the 1st and 3rd periods ($P > 0.05$). At the same dietary taurine level, WGR, SGR, FE, and FR at the 1st period were significantly higher than at the 2nd and 3rd periods ($P < 0.05$). Both dietary taurine level and growth period, as well as their interaction, significantly affected these four parameters ($P < 0.05$).

To determine taurine requirements at different growth stages, regression equations between WGR and dietary taurine level were established for each period

. Optimal dietary taurine levels were 1.20% (1st period, body weight: 13.83–39.09 g), 1.08% (2nd period, body weight: 39.09–66.88 g), and 1.00% (3rd period, body weight: 66.88–101.03 g). These results demonstrate that taurine requirement is closely related to fish age and decreases as fish age increases.

2.2 Effects of Dietary Taurine Level on Morphological Indices Two-way ANOVA using feeding days and dietary taurine level as factors revealed significant effects on morphological indices and . On days 28, 56, and 84, HSI and VSI in taurine groups were significantly lower than in the non-taurine group ($P < 0.05$), while CF showed no significant difference ($P > 0.05$). No significant differences in HSI, VSI, or CF were observed among taurine groups ($P > 0.05$). At the same dietary taurine level, HSI and VSI on days 56 and 84 were significantly lower than on day 28 ($P < 0.05$). Dietary taurine level, feeding days, and their interaction significantly affected HSI and VSI ($P < 0.05$), whereas CF was only significantly affected by feeding days ($P < 0.05$).

2.3 Effects of Dietary Taurine Level on Body Composition on Day 84 Whole-body composition at the end of the trial is shown in . Compared with the non-taurine group, taurine-supplemented groups exhibited significantly higher crude protein content and significantly lower crude lipid content ($P < 0.05$), with no significant differences among taurine groups ($P > 0.05$). No significant differences were observed in moisture or crude ash content among all groups ($P > 0.05$).

Discussion

Numerous studies have demonstrated that dietary taurine significantly promotes fish growth [3,10]. In this trial, taurine-deficient diets resulted in poor growth performance in grouper, consistent with findings in Japanese flounder [15–16], yellowtail [17] (*Seriola quinqueradiata*), red sea bream [18] (*Pagrus major*), and grass carp [3]. The effect of dietary taurine level on grouper growth performance was consistent across all three periods, with WGR increasing then decreasing as taurine level increased , similar to results in rainbow trout [19] and turbot [20]. This may be because juvenile grouper possess relatively strong taurine synthesis capacity [21], while excessive taurine supplementation may reduce feed palatability and intake [22–23], ultimately decreasing growth rate. This trial used 28-day intervals to continuously measure growth performance across three periods, revealing that growth rate was fastest at the 1st period, followed by the 2nd and 3rd periods , consistent with the general pattern of fish development. Using WGR as the evaluation index, regression analysis determined optimal dietary taurine levels of 1.20% (1st period, body weight: 13.83–39.09 g), 1.08% (2nd period, body weight: 39.09–66.88 g), and 1.00% (3rd period, body weight: 66.88–101.03 g) . These findings indicate that taurine requirement decreases with fish age, suggesting that as animals approach adulthood, metabolic processes stabilize and nutrient requirements relatively decline. Although growth

rate gradually decreased from the 1st to 3rd period, all three periods represent relatively fast growth phases in grouper culture.

Taurine exhibits feeding stimulant effects in fish [23], with confirmed positive effects on feed intake in multiple species [19-20,24-29]. This trial demonstrated that FR in taurine-supplemented groups was significantly higher than in the non-taurine group at each growth period, peaking at 1.0% dietary taurine, indicating strong feeding stimulation in juvenile grouper. The non-taurine group showed markedly lower FR and FE than taurine groups, with no further increase in FR and FE as taurine level increased from 0.5% to 1.5%, suggesting that taurine deficiency impairs feed intake and nutrient utilization [30]. Regardless of dietary taurine level, FR and FE gradually decreased across the three periods, contributing to the progressive decline in WGR.

Research shows that taurine promotes lipid digestion, absorption, and metabolism [31]. This trial found that taurine supplementation significantly reduced whole-body crude lipid content, consistent with results in rainbow trout [4], turbot [6], and Atlantic salmon [5], but contrary to findings in grass carp [3] and tilapia [7-8]. Studies have reported that dietary taurine increases hepatic lipase and lipoprotein lipase activities while reducing hepatic triglyceride content in quail [32] and broilers [33]. Although this trial did not measure key lipid metabolism enzymes in grouper liver, the significant reduction in HSI and VSI with taurine supplementation suggests decreased fat deposition in liver and other viscera, which likely accounts for the reduced whole-body lipid content. Taurine supplementation also significantly increased whole-body crude protein content, indicating enhanced protein deposition in juvenile grouper, consistent with reports in Senegalese sole [34] (*Solea senegalensis*), grass carp [3], and common dentex [35] (*Dentex dentex*), possibly through stimulating protein synthesis-related hormone secretion [36]. Regardless of dietary taurine level, HSI and VSI on day 28 were significantly higher than on days 56 and 84, with no significant difference between the latter two, suggesting that after a period of rapid development, organ and tissue growth approaches adult levels and development enters a relatively stable phase.

Conclusions

Appropriate dietary taurine level benefits growth of juvenile grouper, while deficiency or excess is detrimental. Dietary taurine supplementation reduces body lipid deposition while increasing protein deposition in juvenile grouper.

Based on WGR as the evaluation index, regression analysis determined optimal dietary taurine levels of 1.20% (1st period, body weight: 18.83-39.09 g), 1.08% (2nd period, body weight: 39.09-66.88 g), and 1.00% (3rd period, body weight: 66.88-101.03 g) for juvenile grouper, demonstrating that taurine requirements vary among different juvenile stages and gradually decrease with fish age.

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