Effects of dietary methionine supplementation in low-fish-meal diets on growth performance, body composition, and muscle amino acid composition of cobia (Rachycentron canadum) postprint

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Abstract

This experiment aimed to investigate the effects of methionine supplementation in low-fishmeal diets on growth performance, body composition, and muscle amino acid composition of cobia (Rachycentron canadum). DL-methionine was added to low-fishmeal diets to formulate seven isonitrogenous and isolipidic diets with methionine levels of 0.72%, 0.90%, 1.00%, 1.24%, 1.41%, 1.63%, and 1.86%, respectively. A total of 840 cobia with an initial body weight of (9.79$±$0.04) g were randomly divided into seven groups with three replicates per group and 40 fish per replicate, and subjected to a 16-week feeding trial. The results showed that the survival rates in the 0.90% and 1.00% groups were significantly higher than that in the 0.72% group ($P<0.05$). With increasing dietary methionine levels, the weight gain rate, specific growth rate, and protein efficiency of cobia exhibited a trend of initially increasing and then decreasing, reaching maximum values in the 1.00% group, which were significantly higher than those of all other groups ($P<0.05$). The feed conversion ratio was lowest in the 1.00% group, which was not significantly different from the 0.90% and 1.24% groups ($P>0.05$) but significantly lower than all remaining groups ($P<0.05$). The whole-body crude protein content in the 0.72% group was significantly lower than that of all other groups ($P<0.05$); the whole-body crude lipid content peaked in the 0.90% group, which was not significantly different from the 0.72% and 1.00% groups ($P>0.05$) but significantly higher than all remaining groups ($P<0.05$); the whole-body crude ash content in the 1.24% group was significantly higher than that in the 0.72% and 0.90% groups ($P<0.05$). With increasing dietary methionine levels, the contents of phenylalanine, lysine, leucine, alanine, methionine, essential amino acids, and total amino acids in cobia muscle showed no significant differences ($P>0.05$); however, the contents of threonine, valine, isoleucine, and histidine in the 1.00% group were significantly higher than those in the 1.63% group ($P<0.05$). These results indicate that methionine supplementation in low-fishmeal diets can improve the growth performance and body protein content of cobia; using weight gain rate as the evaluation indicator, quadratic regression analysis revealed that the dietary methionine requirement for cobia is 1.12% (accounting for 2.43% of dietary protein).

Full Text

Effects of Methionine Supplementation in Low Fish Meal Diet on Growth Performance, Body Composition and Muscle Amino Acid Composition of Cobia (Rachycentron canadum)

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Abstract: A 16-week feeding trial was conducted to investigate the effects of methionine supplementation in low fish meal diets on growth performance, body composition, and muscle amino acid composition of juvenile cobia (*Rachycentron canadum*). Seven isonitrogenous and isolipidic experimental diets were formulated with graded levels of DL-methionine, yielding dietary methionine concentrations of 0.72%, 0.90%, 1.00%, 1.24%, 1.41%, 1.63%, and 1.86%. A total of 840 juvenile cobia with an initial body weight of (9.79$±$0.04) g were randomly distributed into 7 groups with 3 replicates each (40 fish per replicate). The results showed that survival rates in the 0.90% and 1.00% methionine groups were significantly higher than in the 0.72% group ($P<0.05$). Weight gain rate (WGR), specific growth rate (SGR), and protein efficiency ratio (PER) increased initially and then decreased with rising dietary methionine levels, peaking in the 1.00% group, which was significantly higher than all other groups ($P<0.05$). The feed conversion ratio (FCR) was lowest in the 1.00% group, showing no significant difference from the 0.90% and 1.24% groups ($P>0.05$) but was significantly lower than the remaining groups ($P<0.05$). Whole-body crude protein content in the 0.72% group was significantly lower than in all other groups ($P<0.05$). The highest whole-body crude lipid content was observed in the 0.90% group, which did not differ significantly from the 0.72% and 1.00% groups ($P>0.05$) but was significantly higher than the other groups ($P<0.05$). Whole-body ash content in the 1.24% group was significantly higher than in the 0.72% and 0.90% groups ($P<0.05$). Dietary methionine level had no significant effect on muscle phenylalanine, lysine, leucine, alanine, methionine, essential amino acid (EAA), or total amino acid (TAA) contents ($P>0.05$). However, threonine, valine, isoleucine, and histidine contents in muscle were significantly higher in the 1.00% group compared to the 1.63% group ($P<0.05$). These findings demonstrate that methionine supplementation in low fish meal diets can improve growth performance and body protein content in cobia. Based on quadratic regression analysis using WGR as the response criterion, the optimal dietary methionine requirement for cobia was estimated to be 1.12% of diet (2.43% of dietary protein).

Keywords: cobia; methionine; growth performance; body composition; muscle amino acid content

Fish meal has long served as a high-quality protein source for aquatic animals due to its high protein content, rich essential amino acid profile, abundant long-chain ω-3 fatty acids, and comprehensive vitamin and mineral composition [1]. However, limited fish meal resources and escalating prices in recent years have necessitated reduced usage in aquafeeds [2-3]. Common plant protein sources used as fish meal replacements, such as soybean meal, peanut meal, and cottonseed meal, often lack or contain insufficient essential amino acids, leading to amino acid imbalances that impair efficient protein and amino acid utilization and affect metabolic processes in farmed aquatic animals [4-5]. When fish meal is replaced by plant proteins, methionine typically becomes the first limiting amino acid for normal fish growth [6-7]. In animals, methionine donates active methyl groups to nucleic acids and phospholipids in the form of S-adenosylmethionine, enhancing membrane fluidity and Na^+-K^+-ATPase activity, reducing bile acid accumulation in the liver, and strengthening detoxification functions [8]. Methionine deficiency reduces growth and protein efficiency [9-10], causes appetite loss, growth retardation or stasis, kidney enlargement, hepatic iron accumulation, and can even lead to liver necrosis or fibrosis [11], while also affecting muscle quality and antioxidant capacity [12]. Salmonid species such as rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon, and lake trout (*Salvelinus namaycush*) fed methionine-deficient diets also develop cataracts [13]. Optimal dietary methionine levels improve weight gain, feed utilization, and immune responses in various aquaculture species including grouper (*Epinephelus coioides*) [14], cobia [7,15], Jian carp (*Cyprinus carpio* var. Jian) [8], turbot (*Psetta maxima*) [16], and rainbow trout [17].

Cobia is a promising marine aquaculture species for offshore cage culture systems, with major production in China, Panama, and Vietnam [18-19]. Total production reached approximately 44,000 tons in 2016 [20]. Currently, cobia farming still relies partially on trash fish, limiting large-scale production. This study investigated the effects of supplementing different methionine levels in low fish meal diets on growth performance, body composition, and muscle amino acid composition of cobia to provide theoretical support for formulating efficient practical feeds.

1.1 Experimental Diets and Design

A low fish meal diet containing 20% fish meal was formulated using brown fish meal, dehulled soybean meal, corn gluten meal, wheat gluten flour, and crystalline amino acids (essential and non-essential) as primary protein sources, with soybean oil, fish oil, and soybean lecithin as lipid sources. Graded levels of DL-methionine (0, 0.20%, 0.40%, 0.80%, 1.00%, and 1.20%) were supplemented to create seven isonitrogenous and isolipidic experimental diets (Table 1), with glycine content adjusted accordingly. Feed ingredients were ground, weighed according to formulation, mixed stepwise, and extruded into floating pellets of two sizes (Φ2.5 mm$×5.0mmandΦ4.0mm×$5.0 mm). The diets were air-dried and stored at -20°C until use. The amino acid composition of experimental diets is presented in Table 2 .

1.2 Experimental Fish and Culture Management

The feeding trial was conducted at a fish farm near Nansan Island, Zhanjiang. Juvenile cobia were purchased from a hatchery in Wenchang, Hainan Province. Prior to the experiment, fish were acclimated in net cages (6 m$×3m×2m)foroneweek.Healthyfishwithuniformsize(initialweight : 9.79±0.04g)wererandomlyallocatedinto7groups,with3floatingseawaternetcages(1.0m×1.0m×$2.0 m) per group and 40 fish per cage. Fish were hand-fed twice daily (06:00 and 18:00) to apparent satiation (when most fish stopped swimming to the surface to feed). The culture period lasted 16 weeks under the following conditions: water temperature 28-33°C, salinity 27-30, dissolved oxygen concentration >6 mg/L.

1.3 Sample Collection and Analysis

At the end of the trial, fish were fasted for 24 h, anesthetized with eugenol (1:10,000), counted, and weighed. Three fish per cage were randomly selected and stored at -20°C for whole-body proximate composition analysis. Another four fish per cage were dissected to obtain viscera and liver, which were weighed wet for calculation of morphological indices. Dorsal muscle samples were collected in cryovials, snap-frozen in liquid nitrogen, and stored at -80°C for muscle amino acid composition analysis.

Proximate composition of feed ingredients, experimental diets, and fish was determined according to AOAC (1995) [21]. Samples were oven-dried at 105°C to constant weight for moisture determination. Crude protein content was measured by the Kjeldahl method, crude lipid by Soxhlet extraction, and ash content by combustion at 550°C for 5 h after low-temperature carbonization.

1.4 Determination of Amino Acid Composition in Diets and Muscle

Amino acid composition was analyzed using an automatic amino acid analyzer (A300, membraPure, Germany). Freeze-dried feed and muscle samples (50-200 mg, accurate to 0.1 mg) were hydrolyzed in 10 mL of 6 mol/L HCl in 10 mL headspace vials under vacuum for 10 min, then sealed with nitrogen and aluminum foil. Hydrolysis was performed at 105°C for 24 h, followed by dilution to 50 mL with ultrapure water. One mL of the diluted sample was deacidified in a vacuum oven at 60°C, mixed with 1 mL sodium acetate buffer, filtered through a 0.22 m membrane, and injected for analysis.

1.5 Calculation Formulas

- Weight gain rate (WGR, %) = 100 × (final mean weight - initial mean weight) / initial mean weight
- Specific growth rate (SGR, %/d) = 100 × (ln final mean weight - ln initial mean weight) / feeding days
- Protein efficiency ratio (PER) = 100 × (final body weight - initial body weight) / (feed intake × dietary crude protein content)
- Feed conversion ratio (FCR) = dry feed intake / (final body weight - initial body weight)
- Survival rate (SR, %) = 100 × final fish number / initial fish number
- Condition factor (CF, %) = 100 × body weight (g) / body length $(\text{cm})^3$
- Hepatosomatic index (HSI, %) = 100 × liver weight / body weight
- Viscerosomatic index (VSI, %) = 100 × viscera weight / body weight

1.6 Statistical Analysis

Data were analyzed using SPSS 17.0 software. One-way ANOVA was performed, and Duncan' s multiple range test was applied when significant differences were detected ($P<0.05$). Results are expressed as "mean ± standard error."

2.1 Effects of Dietary Methionine Level on Growth Performance of Cobia

As shown in Table 3 , survival rates across groups ranged from 77.15% to 92.86%, with the 0.90% and 1.00% groups showing significantly higher SR than the 0.72% group ($P<0.05$). WGR, SGR, and PER increased initially and then decreased with increasing dietary methionine, reaching maximum values in the 1.00% group, which were significantly higher than all other groups ($P<0.05$). The FCR was lowest in the 1.00% group, showing no significant difference from the 0.90% and 1.24% groups ($P>0.05$) but was significantly lower than the remaining groups ($P<0.05$).

Quadratic regression analysis between WGR (y) and dietary methionine level (x) revealed a significant relationship (Figure 1 [Figure 1: see original paper]): $y = -734.1x^2 + 1{,}644.2x + 200.89$ ($R^2 = 0.627$). The maximum WGR was achieved at a dietary methionine level of 1.12%, with a 95% confidence interval of 0.87%-1.63% estimated from the seven dietary methionine levels.

2.2 Effects of Dietary Methionine Level on Morphological Parameters of Cobia

As presented in Table 4 , the condition factor (CF) in the 1.41% group was significantly lower than in the 0.72% group ($P<0.05$) but did not differ significantly from other groups ($P>0.05$). Dietary methionine level had no significant effects on VSI or HSI ($P>0.05$).

2.3 Effects of Dietary Methionine Level on Whole-Body Composition of Cobia

Table 5 shows that whole-body moisture content did not differ significantly among groups ($P>0.05$). Whole-body crude protein content in the 0.72% group was significantly lower than in all other groups ($P<0.05$). The highest crude lipid content (30.81%) was observed in the 0.90% group, which was not significantly different from the 0.72% and 1.00% groups ($P>0.05$) but was significantly higher than the remaining groups ($P<0.05$). Whole-body ash content in the 1.24% group was significantly higher than in the 0.72% and 0.90% groups ($P<0.05$).

2.4 Effects of Dietary Methionine Level on Muscle Amino Acid Composition of Cobia

With increasing dietary methionine levels, muscle contents of phenylalanine, lysine, leucine, alanine, methionine, essential amino acids (EAA), and total amino acids (TAA) showed no significant changes ($P>0.05$). However, threonine, valine, isoleucine, and histidine contents in muscle were significantly higher in the 1.00% group compared to the 1.63% group ($P<0.05$).

3.1 Effects of Dietary Methionine Level on Growth Performance and Morphological Indices

In this study, feeding a methionine-deficient diet (0.72% methionine) reduced growth and survival in cobia, consistent with observations in grouper [14], black seabream (*Sparus macrocephalus*) [22], Indian catfish (*Heteropneustes fossilis*) [23], and blunt snout bream (*Megalobrama amblycephala*) [24]. Methionine deficiency impairs protein synthesis and may reduce antioxidant products, causing irreversible oxidative stress that exacerbates growth stasis [9]. As dietary methionine increased to 1.00%, growth performance and feed utilization improved significantly, indicating that supplementing methionine to optimal levels in low-fish-meal diets enhances cobia growth. Based on WGR, the methionine requirement for cobia was estimated at 1.12% of diet (2.43% of dietary protein), which promotes growth and improves feed efficiency. This requirement as a percentage of dietary protein is similar to previous values for cobia (2.64%) [7], yellowtail (2.56%) [25], and blunt snout bream (2.47%-2.50%) [24], but lower than black seabream (4.50%-4.53%) [22], European seabass (4.4%) [26], yellow catfish (3.48%-3.53%) [9], large yellow croaker (3.22%-3.34%) [27], yellow perch (3.10%-3.40%) [28], and mrigal carp (3.0%) [6], and higher than rainbow trout

(1.49%) [29] and gibel carp (2.17%) [30]. These discrepancies may arise from differences in species, size, diet formulation, culture management, and environmental conditions. For instance, the crude protein content in this study (46%) was higher than in studies on rainbow trout (35%) [29] and gibel carp (37%) [30]. Additionally, methionine absorption, supplementation form, and bioavailability may affect requirements [31-32].

When dietary methionine reached or exceeded 1.24%, WGR, SGR, and PER gradually decreased, consistent with findings in blunt snout bream [24] and cobia [15]. Imbalanced dietary amino acid patterns can reduce growth and protein deposition while increasing nitrogen excretion and amino acid oxidation [33]. Excess methionine may accumulate and be oxidized, producing harmful metabolites. During decarboxylation, amino acids generate primary amines, most of which are toxic to animals. Amines are oxidized by amine oxidases to aldehydes and ammonia, which must be excreted [34-36]. Excess methionine deamination also consumes additional energy, reducing PER [24]. However, some studies reported that WGR increased significantly up to the optimal methionine level and then remained stable [14,37-38].

Dietary cysteine content may influence methionine requirements. Previous studies showed that cysteine can spare methionine by 60% in channel catfish [38], 40%-50% in red drum [39], 49% in Nile tilapia [37], and 39.6%-40.2% in Indian catfish [40]. Therefore, separate consideration of methionine and cysteine requirements is recommended during diet formulation to ensure adequate provision of both amino acids [41]. However, since many feed ingredients contain more cysteine than methionine, practical diets often focus only on methionine requirements [42]. The conversion efficiency of methionine to cysteine and the sparing ratio in cobia remain unclear and warrant further investigation.

Dietary methionine level had no significant effect on HSI or VSI in this study, consistent with previous cobia research [7]. However, other studies on yellow catfish [9], rainbow trout [43], and Atlantic salmon [44] found significantly higher HSI in fish fed methionine-deficient diets compared to those fed excess methionine. Conversely, lower HSI was observed in grouper [14] and rockfish [45] fed methionine-deficient diets. These inconsistent results suggest species-specific responses to dietary methionine levels.

3.2 Effects of Dietary Methionine Level on Body Composition

Whole-body crude protein content reportedly increases initially and then decreases with rising dietary methionine levels [6,40,45]. In this study, cobia fed the methionine-deficient diet had significantly lower whole-body crude protein content. This may be because methionine deficiency creates an imbalanced amino acid pattern, limiting utilization of other amino acids, increasing deamination of excess amino acids, and ultimately restricting body protein synthesis [46]. Supplementing methionine enhanced utilization of other amino acids and promoted protein synthesis. Luo et al. [14] reported that whole-body crude protein in grouper increased with methionine up to the optimal level and then stabilized.

Interestingly, cobia fed low-methionine diets had higher whole-body crude lipid content, consistent with results in blunt snout bream [24], Indian catfish [40], and Nile tilapia [47]. This suggests that fish fed methionine-deficient diets may utilize protein rather than lipid as an energy source, reducing long-chain acyl-CoA transport from the cytosol to mitochondria for β-oxidation [24,48]. However, other studies reported increased whole-body lipid content with rising methionine levels [14,23,25], conflicting with these results and requiring further investigation.

3.3 Effects of Dietary Methionine Level on Muscle Amino Acid Composition

Aquatic animals accumulate protein through dietary amino acid synthesis, and different dietary amino acid patterns affect growth, body protein-bound amino acid composition, and protein synthesis [27,49]. Previous studies reported that muscle EAA content in yellow catfish [10], muscle EAA, non-essential amino acid (NEAA), and TAA contents in bullfrog [50], and whole-body EAA and TAA contents in basa catfish [51] were unaffected by dietary methionine levels. Similarly, this study found no significant effect of dietary methionine on muscle EAA and TAA contents in cobia, contrasting with results in black seabream [22], large yellow croaker [27], and Chinese sucker [52], where methionine deficiency reduced muscle EAA content and inhibited protein synthesis.

Notably, dietary methionine level significantly affected muscle threonine, valine, isoleucine, histidine, and arginine contents, similar to previous findings [14,52]. This indicates that intake of one amino acid can influence the content of others [53], and limitation of one essential amino acid may increase oxidation of other essential and non-essential amino acids to achieve dietary amino acid balance [22].

Conclusions:

1. Methionine supplementation in low fish meal diets can significantly improve growth performance and body protein content in cobia.
2. Based on quadratic regression analysis using weight gain rate as the evaluation criterion, the optimal dietary methionine requirement for cobia is 1.12% of diet (2.43% of dietary protein).

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