Effects of Dietary n-3 Highly Unsaturated Fatty Acids Levels on Growth Performance, Serum Biochemical Indices, and Muscle and Liver Fatty Acid Composition in Larger Size Cobia (Rachycentron canadum) Postprint

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Abstract

This experiment aimed to investigate the effects of dietary n-3 highly unsaturated fatty acids (HUFA) levels on growth performance, serum biochemical indices, and muscle and liver fatty acid composition of larger-sized cobia (Rachycentron canadum), in order to determine the optimal dietary n-3HUFA level for larger-sized cobia. Using white fish meal, casein, and dehulled soybean meal as the main protein sources, and adjusting the addition levels of fish oil and corn oil in the diets, seven isonitrogenous and isoenergetic experimental diets with n-3HUFA levels of 0.49%, 0.73%, 0.98%, 1.41%, 1.51%, 2.06%, and 2.83% were formulated and fed to cobia with an initial weight of 70 g for 8 weeks. Each diet was fed to three cages (replicates), with each cage stocked with 30 fish. The results showed: 1) Weight gain rate (WGR) and specific growth rate (SGR) showed a trend of first increasing then decreasing with increasing dietary n-3HUFA levels, with the 0.73%–1.51% groups being significantly higher than the 0.49% and 2.83% groups ($P<0.05$). The survival rate (SR) of the 2.83% group was significantly lower than other groups ($P<0.05$). Whole-body crude lipid content showed a trend of first increasing then decreasing with increasing dietary n-3HUFA levels, with the 0.98% and 1.41% groups being significantly higher than the 2.06% and 2.83% groups ($P<0.05$). 2) Serum alanine aminotransferase (ALT) activity in the 0.49% group was significantly higher than that in the 2.06% group ($P<0.05$); serum triglycerides (TG) in the 0.49% group were significantly higher than other groups ($P<0.05$); serum total cholesterol (CHOL) content showed a trend of first increasing then decreasing with increasing dietary n-3HUFA levels, being highest in the 0.73% group and significantly higher than the 0.49%, 2.06%, and 2.83% groups ($P<0.05$); serum high-density lipoprotein (HDLC) content in the 0.98% group was significantly higher than other groups (P<0.05); serum low-density lipoprotein (LDLC) content in the 0.98%-2.83% groups was significantly higher than the 0.49% and 0.73% groups (P<0.05). 3) Muscle and liver C14:0 and C21:0 contents were highest in the 2.83% group, while there were no significant differences in muscle and liver C16:0 content and total saturated fatty acids (SFA) among groups (P>0.05). Muscle and liver C18:1n-9, total monounsaturated fatty acids (MUFA), and C18:2n-6 contents all decreased with increasing dietary n-3HUFA levels. With increasing dietary n-3HUFA levels, total n-6 polyunsaturated fatty acids (n-6PUFA) in muscle and liver showed a trend of first increasing then stabilizing, while C20:5n-3 (EPA), C22:5n-3, C22:6n-3 (DHA) contents, total n-3 polyunsaturated fatty acids (n-3PUFA), and total n-3HUFA (n-3HUFA) all showed a gradually increasing trend. In conclusion, dietary supplementation of n-3HUFA helps improve growth performance, reduce body fat deposition, promote lipid metabolism, and affect muscle and liver fatty acid composition in larger-sized (70 g) cobia. Using SGR as the evaluation index, the broken-line model indicated that the optimal dietary n-3HUFA level for larger-sized (70 g) cobia was 0.95%.

Full Text

Effects of Dietary n-3 Highly Unsaturated Fatty Acids Levels on Growth Performance, Serum Biochemical Indices, and Fatty Acid Composition in Muscle and Liver of Larger Cobia (Rachycentron canadum)

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Abstract: This experiment investigated the effects of dietary n-3 highly unsaturated fatty acids (n-3HUFA) on growth performance, serum biochemical indices, and fatty acid composition in muscle and liver of larger cobia (Rachycentron canadum) to determine the optimal dietary n-3HUFA level. Seven isonitrogenous and isoenergetic experimental diets were formulated using white fish meal, casein, and peeled soybean meal as primary protein sources, with varying proportions of fish oil and corn oil to achieve graded n-3HUFA levels of 0.49%, 0.73%, 0.98%, 1.41%, 1.51%, 2.06%, and 2.83% (measured values). Cobia with initial body weight of 70 g were fed these diets for 8 weeks, with three replicate cages per diet and 30 fish per cage. Results showed: (1) Weight gain rate (WGR) and specific growth rate (SGR) increased initially then decreased with rising dietary n-3HUFA levels, with values in the 0.73%–1.51% groups significantly higher than those in the 0.49% and 2.83% groups (P<0.05). Survival rate (SR) in the 2.83% group was significantly lower than other groups (P<0.05). Body crude lipid content showed a similar trend, with the 0.98% and 1.41% groups significantly higher than the 2.06% and 2.83% groups (P<0.05). (2) Serum alanine transaminase (ALT) activity in the 0.49% group was significantly higher than in the 2.06% group (P<0.05). Serum triglyceride (TG) content in the 0.49% group was significantly higher than all other groups (P<0.05). Serum total cholesterol (CHOL) increased then decreased with dietary n-3HUFA level, peaking in the 0.73% group, which was significantly higher than the 0.49%, 2.06%, and 2.83% groups (P<0.05). Serum high-density lipoprotein cholesterol (HDLC) content in the 0.98% group was significantly higher than other groups (P<0.05). Serum low-density lipoprotein cholesterol (LDLC) content in the 0.98%–2.83% groups was significantly higher than in the 0.49% and 0.73% groups (P<0.05). (3) Muscle and liver C14:0 and C21:0 contents were highest in the 2.83% group, while C16:0 content and total saturated fatty acids (SFA) showed no significant differences among groups (P>0.05). C18:1n-9, C18:2n-6, and total monounsaturated fatty acids (MUFA) decreased with increasing dietary n-3HUFA levels. Total n-6 polyunsaturated fatty acids (n-6PUFA) increased initially then plateaued, while C20:5n-3 (EPA), C22:5n-3, C22:6n-3 (DHA), total n-3 polyunsaturated fatty acids (n-3PUFA), and total n-3HUFA (n-3HUFA) all increased gradually (P<0.05). In conclusion, dietary n-3HUFA supplementation improved growth performance, reduced body lipid deposition, enhanced lipid metabolism, and affected muscle and liver fatty acid composition in larger cobia (70 g). Based on SGR, the optimal dietary n-3HUFA level for larger cobia was determined to be 0.95% using a broken-line model.

Key words: larger cobia; n-3 highly unsaturated fatty acids; growth performance; serum biochemical indices; fatty acid composition

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Cobia (*Rachycentron canadum*), belonging to the family Rachycentridae, order Perciformes, is widely distributed in tropical and subtropical regions except the eastern Pacific Ocean. As a warm-water fish species primarily cultured in cages, cobia can reach 6–8 kg within one year, representing significant economic value. While nutritional requirements for protein, lipid, vitamins, minerals, and carbohydrates have been reported for juvenile cobia, fish have different nutrient requirements at different growth stages. Investigating these stage-specific requirements is essential for designing precise and efficient formulated feeds, reducing costs, and promoting sustainable aquaculture development.

Polyunsaturated fatty acids (PUFA) contain two or more double bonds with carbon chain lengths of 18–22. n-3 polyunsaturated fatty acids (n-3PUFA) are characterized by the first double bond at the third carbon from the methyl end. n-3 highly unsaturated fatty acids (n-3HUFA) are n-3PUFA with three or more double bonds and carbon chains longer than 20, primarily including eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Numerous studies demonstrate that n-3HUFA plays crucial roles in maintaining cell membrane fluidity, promoting growth, enhancing immunity, improving antioxidant capacity, reducing inflammatory responses, and regulating lipid metabolism. However, marine fish have limited capacity to convert short-chain and less unsaturated fatty acids into n-3HUFA, requiring adequate dietary supplementation for normal growth. n-3HUFA requirements have been reported for various species including Japanese flounder (*Paralichthys olivaceus*), black seabream (*Sparus macrocephalus*), orange-spotted grouper (*Epinephelus coioides*), and European sea bass (*Dicentrarchus labrax*). Although n-3HUFA requirements for cobia have been studied, previous research focused only on juveniles (8.3±0.5 g), with no data available for larger specimens. This study investigated the effects of dietary n-3HUFA levels on growth performance, body composition, serum biochemical indices, and muscle and liver fatty acid composition in larger cobia to determine optimal dietary n-3HUFA levels and provide foundational data for precise feed formulation.

1.1 Experimental Diets and Design

Seven isonitrogenous and isoenergetic experimental diets were formulated with white fish meal, casein, and peeled soybean meal as primary protein sources, wheat flour as carbohydrate source, and fish oil and corn oil as main lipid sources. Dietary n-3HUFA levels were adjusted to 0.49%, 0.73%, 0.98%, 1.41%, 1.51%, 2.06%, and 2.83% (measured values) by varying fish oil and corn oil proportions . Feed ingredients were ground through a 60-mesh sieve, weighed according to formulation, and micro-components were mixed using the progressive expansion method. Fish oil, corn oil, and soybean lecithin oil were added and mixed evenly, followed by blending in a V-type vertical mixer for 5 minutes. Water (30%–40% by weight) was added before extrusion into 3.0 mm pellets using an F-26 twin-screw extruder (South China University of Technology, Guangzhou). Pellets were air-dried in a ventilated dark place to approximately 10% moisture, sealed in bags, and stored at -20°C. The fatty acid composition of experimental diets is shown in .

1.2 Experimental Animals and Culture Management

Cobia juveniles were obtained from Sanya, Hainan Province, as a single batch of artificially propagated offspring. The feeding trial was conducted in offshore floating cages in Zhanjiang, Guangdong Province. Prior to the experiment, cobia were acclimated in 4.5 m × 4.5 m × 3.0 m cages for three weeks with chopped fresh fish, then trained for two weeks with commercial feed (Guangdong Yuejia Feed Co., Ltd., 42% crude protein) to reach the required size. After 24 h fasting, healthy cobia with uniform size (initial weight ~70 g) were randomly distributed into seven groups with three replicates each (30 fish per replicate) in 1.4 m × 1.9 m × 2.5 m cages. The 8-week feeding trial involved feeding to satiation twice daily (09:00 and 17:00). Water temperature ranged 29.0–32.5°C, salinity 26–29, pH 7.5–7.8, ammonia nitrogen <0.28 mg/L, and dissolved oxygen

6–7 mg/L.

1.3 Sample Collection and Analysis

At the end of the trial, fish were fasted for 24 h, anesthetized with eugenol, and weighed and counted per cage for growth calculation. Five fish per cage were frozen for whole-body composition analysis. Another five fish per cage were randomly selected for blood collection from the heart using 2 mL syringes. Blood samples were placed in anticoagulant-free tubes, left at room temperature for 4 h, then centrifuged (5,000×g, 10 min, 4°C). Serum was collected and stored at -80°C for biochemical analysis. After blood collection, liver and dorsal muscle samples were rapidly frozen in liquid nitrogen and stored at -80°C for fatty acid analysis.

1.4 Analytical Methods

Moisture content in feed and whole fish was determined by oven drying at 105°C to constant weight. Crude protein was measured by the Kjeldahl method (Kjeltec™ 8400, Sweden), crude lipid by Soxhlet extraction with ether, and crude ash by muffle furnace incineration at 550°C. Serum total protein (TP), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) were analyzed using an automatic biochemical analyzer (Hitachi 7600-110). Fatty acid composition was determined by gas chromatography (Varian 430-GC, Agilent) with an HP-88 column (60 m × 0.25 mm × 0.2 m). The FID detector and injector temperatures were 260°C with a split ratio of 30:1. The temperature program started at 140°C for 5 min, increased at 5°C/min to 240°C, and held for 20 min. Column flow was 2 mL/min with nitrogen as carrier gas (25 mL/min) and hydrogen as fuel gas (30 mL/min). Fatty acids were identified using standard methyl esters and quantified by area normalization.

1.5 Calculation Formulas

Survival rate (SR) = 100 × Nt/N0
Weight gain rate (WGR) = 100 × (Wt -W0)/W0
Specific growth rate (SGR) = 100 × (lnWt -lnW0)/t
Condition factor (CF) = 100 × Wt/L^3
Feed conversion ratio (FCR) = Id/(Wt -W0)

Where: Wt = final body weight (FBW) (g); W0 = initial body weight (IBW) (g); t = experimental duration (d); N0 = initial fish number; Nt = final fish number; Id = total dry feed intake (g); L = body length (cm).

1.6 Statistical Analysis

Data are presented as mean ± standard error (SE). One-way ANOVA was performed using SPSS 17.0 software. When significant differences were detected

(P<0.05), Duncan' s multiple range test was used for pairwise comparisons.

2.1 Effects of Dietary n-3HUFA Level on Growth Performance of Larger Cobia

As shown in , WGR and SGR in the 0.73%-1.41% groups were significantly higher than in all other groups except the 1.51% group (P<0.05), while no significant differences were observed among the 0.49%, 2.06%, and 2.83% groups (P>0.05). Survival rate in the 2.83% group was significantly lower than all other groups (P<0.05), with no significant differences among the remaining groups (P>0.05). Condition factor and feed conversion ratio were not significantly affected by dietary n-3HUFA level (P>0.05), though the lowest numerical values occurred in the 1.41% group. Using SGR as the criterion, broken-line model analysis [Figure 1: see original paper] yielded linear equations y=0.575x+1.7978(R^2=0.8003) and y=-0.1659x+2.4918(R^2=0.8994), indicating maximum SGR at a dietary n-3HUFA level of 0.95%.

2.2 Effects of Dietary n-3HUFA Level on Body Composition of Larger Cobia

As shown in , crude protein, moisture, and ash contents were not significantly affected by dietary n-3HUFA level (P>0.05). Body crude lipid content increased initially then decreased with rising dietary n-3HUFA levels, with the 2.83% group significantly lower than all groups except 2.06% (P<0.05), and the 0.98% and 1.41% groups significantly higher than the 2.06% group (P<0.05). No significant differences were observed among the 0.49%-1.51% groups (P>0.05).

2.3 Effects of Dietary n-3HUFA Level on Serum Biochemical Indices of Larger Cobia

As shown in , serum ALT activity in the 2.06% group was significantly lower than in the 0.49% group (P<0.05) but did not differ significantly from other groups (P>0.05). Serum TG content decreased fluctuatingly with increasing dietary n-3HUFA level, with the 0.49% group significantly higher than all others (P<0.05), the 0.73% group significantly lower than 0.49% but higher than remaining groups (P<0.05), and no significant differences among the 1.51%-2.83% groups (P>0.05), though these were significantly lower than all groups except 0.98% (P<0.05). Serum CHOL content increased then decreased with dietary n-3HUFA level, peaking in the 0.73% group, which was significantly higher than the 0.49%, 2.06%, and 2.83% groups (P<0.05). Serum HDLC and LDLC contents both increased initially then declined. HDLC content in the 0.98% group was significantly higher than all other groups (P<0.05), while the 1.41% group was significantly lower than the 0.98% and 2.83% groups (P<0.05) but not different from remaining groups (P>0.05). LDLC content in the 0.49% and 0.73% groups was significantly lower than other groups (P<0.05), with no significant difference between the 1.51% and 2.06% groups (P>0.05), though both were significantly higher than other groups ($P<0.05$). The 2.83% group showed significantly lower LDLC than the 2.06% group ($P<0.05$).

2.4 Effects of Dietary n-3HUFA Level on Fatty Acid Composition in Muscle and Liver of Larger Cobia

As shown in , muscle C14:0 and C18:0 contents in the 0.73%-2.83% groups did not differ significantly from the 0.49% group ($P>0.05$), while the 2.83% group showed the highest values, with C14:0 significantly higher than the 1.41% and 1.51% groups ($P<0.05$) and C18:0 significantly higher than the 0.73% and 0.98% groups ($P<0.05$). Muscle C21:0 content was highest in the 2.83% group, significantly exceeding all other groups ($P<0.05$). No significant differences were observed among groups in total saturated fatty acids (SFA) or C16:0 content ($P>0.05$), with C16:0 being the most abundant saturated fatty acid. In muscle monounsaturated fatty acids, C18:1n-9 was the most abundant, and total monounsaturated fatty acids (MUFA) decreased with increasing dietary n-3HUFA level. Muscle C16:1n-9 and C18:1n-7 contents in the 2.06%-2.83% groups were higher than the 0.49% group but not significantly different ($P>0.05$). Total n-6 polyunsaturated fatty acids (n-6PUFA) in the 1.51%-2.83% groups were significantly higher than other groups ($P<0.05$). Muscle C18:2n-6 content decreased while C20:4n-6 content increased then decreased with rising dietary n-3HUFA levels. EPA and DHA contents increased gradually, with significant differences among all groups except between 0.49% and 0.73% for EPA ($P>0.05$). DHA/EPA ratios increased significantly compared to the 0.49% group ($P<0.05$).

As shown in , liver total saturated fatty acids, C16:0, and C21:0 showed similar trends to muscle, with C14:0 increasing significantly and peaking at 2.83% ($P<0.05$). Liver C18:0 content remained relatively stable, though the 2.06% group was significantly lower than the 0.49% group ($P<0.05$). Liver C16:1n-9 content was highest in the 2.06% group, significantly exceeding the 0.49% and 0.73% groups ($P<0.05$). Liver C18:1n-7 content increased significantly with dietary n-3HUFA level ($P<0.05$), while C18:1n-9 content and MUFA decreased. Liver n-6PUFA in the 1.51%-2.83% groups was significantly higher than other groups ($P<0.05$). Liver C20:4n-6 content increased significantly ($P<0.05$) while C18:2n-6 content decreased continuously with rising dietary n-3HUFA levels. Liver n-3PUFA and DHA/EPA trends matched those in muscle, with the 2.83% group showing significantly lower DHA/EPA than the 2.06% group ($P<0.05$).

In both muscle and liver, C16:0, C18:1n-9, and C18:2n-6 were the most abundant fatty acids, followed by C18:0, DHA, and EPA, generally reflecting dietary fatty acid profiles. Muscle contents of n-6PUFA, n-3PUFA, and n-3HUFA were higher than in liver, while MUFA was lower. Except for the 2.83% group, DHA/EPA ratios were higher in muscle than in liver.

3 Discussion

The present study demonstrated that dietary n-3HUFA levels of 0.73%-1.51% significantly improved WGR and SGR compared to the 0.49% group in larger cobia (70 g), with broken-line model analysis indicating an optimal requirement of 0.95% based on SGR. Reported n-3HUFA requirements vary among species: 0.7% for European sea bass (14 g), 1.27%-1.42% for orange-spotted grouper (50 g), 0.87% for black seabream (8.08±0.09 g), 1.0% for gilthead seabream (11.5±0.2 g), and 0.8%-1.0% for Japanese flounder (8.5±0.06 g). The 0.95% requirement for larger cobia is lower than the 1.49% reported for juvenile cobia (8.3±0.5 g), confirming that n-3HUFA requirements vary with developmental stage within the same species. Studies on tench (*Tinca tinca* L.) showed that DHA and EPA contents in adult and broodstock muscle were significantly higher than in juveniles, suggesting stage-specific fatty acid requirements. Similarly, *Litopenaeus vannamei* adults (8.50 g) required less n-3HUFA (0.51%) than juveniles (0.50 g, 0.89%) and subadults (4.25 g, 0.90%). The reduced n-3HUFA requirement in larger cobia may indicate enhanced endogenous synthesis capacity, though the underlying mechanisms require further investigation.

Consistent with previous research, both low (0.49%) and high (2.83%) n-3HUFA levels reduced WGR and SGR, while 0.73%-1.51% n-3HUFA produced optimal growth. The 2.83% group exhibited significantly lower SR, and the 1.41% group showed numerically lower FCR, aligning with reports that appropriate n-3HUFA levels promote growth while deficiency or excess impairs performance and feed utilization. Condition factor was not significantly affected, consistent with findings in grouper and black seabream juveniles.

Body crude protein, moisture, and ash contents were unaffected by dietary n-3HUFA level, while crude lipid content showed a significant quadratic response. This contrasts with some studies reporting no significant effects on body composition in European sea bass, black seabream, grouper, and juvenile cobia. However, our findings agree with research showing decreased body lipid with high dietary n-3HUFA in black seabream juveniles, Japanese flounder eggs, and Japanese seabass. Conversely, EPA and DHA increased muscle lipid in Japanese flounder juveniles, and high n-3HUFA elevated lipid content in swimming crab (*Portunus trituberculatus*) juveniles and Japanese seabass liver. These discrepancies suggest that n-3HUFA regulates lipid metabolism in a species- and tissue-specific manner.

Serum biochemical indices reflect fish health, nutritional status, and metabolism. ALT is a key liver function marker; elevated serum ALT typically indicates tissue damage. The highest ALT activity in the 0.49% group suggests that n-3HUFA deficiency may impair liver health, while adequate levels maintain normal function. This aligns with reports that inappropriate n-3HUFA levels elevate ALT in large yellow croaker (*Larmichthys crocea*) and that optimal levels reduce ALT in *L. vannamei*. Serum TG and CHOL are important lipid metabolism indicators. The overall decline in TG and CHOL with increasing n-3HUFA (except for the CHOL peak at 0.73%) agrees with findings in Amur sturgeon (*Acipenser schrenckii*). The CHOL peak at 0.73% may reflect the absence of fish oil (rich in cholesterol) in the 0.49% diet. Contradictory results showing increased CHOL with n-3HUFA in black seabream and marbled rockfish (*Sebastiscus marmoratus*) highlight the need for further research on n-3HUFA' s cholesterol-regulating mechanisms. HDLC transports excess CHOL to the liver for catabolism, while LDLC delivers CHOL to peripheral tissues. The elevated HDLC and LDLC at moderate n-3HUFA levels (0.98%-2.06%) suggest enhanced lipid utilization and metabolic capacity, though this pattern differs from *L. vannamei*, possibly due to species-specific metabolism.

Dietary n-3HUFA levels were manipulated by adjusting fish oil and corn oil proportions, similar to previous cobia studies using fish oil and tallow. The predominance of C16:0, C18:1n-9, C18:2n-6, DHA, and EPA in cobia tissues matches reports in European sea bass, spotted grunt (*Plectorhynchus cinctus*), grouper, and juvenile cobia, underscoring their biological importance. The increasing muscle and liver n-3PUFA and n-3HUFA with dietary level confirms efficient absorption. Stable SFA reflects consistent C16:0 and C18:0 contents, which serve as primary energy sources and membrane components that can be endogenously synthesized. The decreasing MUFA, driven by C18:1n-9, suggests its preferential utilization as an energy substrate over SFA and n-3PUFA, consistent with reports that MUFA are preferentially catabolized during starvation in yellow catfish (*Pelteobagrus fulvidraco*) larvae. The contrasting trends of decreasing C18:2n-6 and increasing n-6PUFA require further mechanistic study. The significant increase in DHA and EPA with dietary n-3HUFA and the plateauing DHA/EPA ratio in muscle at high n-3HUFA levels (1.41%-2.83%) suggests robust DHA accumulation capacity in muscle relative to liver, consistent with previous cobia research but differing from black seabream. The higher SFA, n-6PUFA, n-3PUFA, and n-3HUFA but lower MUFA in muscle compared to liver, along with preferential DHA and EPA storage in muscle, contrasts with crustacean patterns where energy fatty acids concentrate in hepatopancreas while essential fatty acids are stored in muscle.

In conclusion, optimal dietary n-3HUFA levels (0.95%) enhanced growth, reduced lipid deposition, improved lipid metabolism, and modulated fatty acid utilization and accumulation in larger cobia (70 g). These findings provide essential data for developing stage-specific precise feeds for cobia aquaculture.

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