Postprint: Dietary n-3 Highly Unsaturated Fatty Acid Requirement in Sillago sihama

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the dietary n-3 highly unsaturated fatty acid (HUFA) requirement of Sillago sihama. A total of 540 juvenile Sillago sihama with an average weight of (6.0±0.6) g were selected and randomly divided into 6 groups, with 3 replicates per group and 30 fish per replicate. Each group was fed isonitrogenous and isolipidic diets containing n-3HUFA levels of 0.53%, 0.80%, 1.22%, 1.62%, 2.12%, and 2.56% for 8 weeks. The results showed: 1) The 2.12% group exhibited the highest weight gain rate and specific growth rate, which were significantly higher than those of the 0.53% group ($P<0.05$); the 2.12% group showed the lowest feed conversion ratio, which was significantly lower than that of the 0.53% group ($P<0.05$); the 2.12% group had the lowest hepatosomatic index, which was significantly lower than those of the 0.53% and 0.80% groups ($P<0.05$); the 2.12% group demonstrated the highest condition factor, which was significantly higher than those of all other groups except the 1.62% group ($P<0.05$). 2) No significant differences were observed in whole-body moisture, crude protein, or crude ash content among all groups ($P>0.05$); the 1.62% group exhibited the highest whole-body crude lipid content, which was significantly higher than those of all other groups except the 1.62% group ($P<0.05$). 3) The serum high-density lipoprotein cholesterol (HDL-C) content in the 2.56% group was significantly higher than those of all other experimental groups except the 2.12% group ($P<0.05$); the serum low-density lipoprotein cholesterol (LDL-C) content in the 0.80% group was significantly higher than those of all other groups ($P<0.05$); the serum alanine aminotransferase (ALT) activity in the 1.22% group was significantly lower than those of all other groups ($P<0.05$); the serum aspartate aminotransferase (AST) activity in the 0.80% group was significantly lower than those of all other groups ($P<0.05$); the serum total cholesterol (T-CHOL) and triglyceride (TG) contents in the 0.53% group were significantly lower than those of all other groups ($P<0.05$); the serum CHOL and TG contents in the 0.80% group were significantly higher than

those of all other groups (P<0.05). 4) No significant differences were observed in serum superoxide dismutase (SOD) and acid phosphatase (ACP) activities among all groups (P>0.05); the serum alkaline phosphatase (AKP) activities in the 2.12% and 2.56% groups were significantly lower than those of all other groups except the 1.62% group (P<0.05); the serum catalase (CAT) activity in the 1.62% group was significantly lower than those of all other groups except the 1.22% and 2.12% groups (P<0.05). 5) With increasing dietary n-3HUFA levels, the hepatic contents of C18:3n-3, C20:5n-3, and C22:6n-3 increased significantly (P<0.05), while the hepatic C18:2n-6 content decreased significantly (P<0.05). 6) With increasing dietary n-3HUFA levels, the muscular C18:2n-6 and C20:4n-6 contents showed a decreasing trend, with the 2.12% and 2.56% groups being significantly lower than all other groups (P<0.05); the muscular C20:5n-3 and C22:6n-3 contents showed an increasing trend, with the 2.56% group being significantly higher than all other groups (P<0.05). These results indicate that based on weight gain rate, the broken-line model fitting revealed that the dietary n-3HUFA requirement for juvenile Sillago sihama was 2.21%.

Full Text

Requirement of Dietary n-3 Highly Unsaturated Fatty Acids of Sillago sihama

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Abstract

This experiment was conducted to investigate the dietary requirement of n-3 highly unsaturated fatty acids (n-3HUFA) for Sillago sihama. A total of 540 juvenile Sillago sihama with an average body weight of (6.0±0.6) g were randomly allocated into six groups with three replicates per group and 30 fish per replicate. The fish were fed isonitrogenous and isolipidic diets containing graded levels of n-3HUFA (0.53%, 0.80%, 1.22%, 1.62%, 2.12%, and 2.56%) for eight weeks. The results showed: 1) The 2.12% group exhibited the highest weight gain rate (WGR) and specific growth rate (SGR), which were significantly higher than those of the 0.53% group (P<0.05). The 2.12% group showed the lowest feed conversion ratio (FCR), significantly lower than the 0.53% group (P<0.05). The hepatosomatic index (HSI) was lowest in the 2.12% group, significantly lower than in the 0.53% and 0.80% groups (P<0.05). The condition factor (CF) was highest in the 2.12% group, significantly higher than all other groups except the 1.62% group (P<0.05). 2) No significant differences were observed among

groups in whole-body moisture, crude protein, or crude ash content (P>0.05). The 1.62% group had the highest whole-body crude lipid content, significantly higher than all other groups except the 2.12% group (P<0.05). 3) Serum high-density lipoprotein cholesterol (HDL-C) content in the 2.56% group was significantly higher than in all other groups except the 2.12% group (P<0.05). Serum low-density lipoprotein cholesterol (LDL-C) content in the 0.80% group was significantly higher than in all other groups (P<0.05). Serum alanine aminotransferase (ALT) activity in the 1.22% group was significantly lower than in all other groups (P<0.05), while serum aspartate aminotransferase (AST) activity in the 0.80% group was significantly lower than in all other groups (P<0.05). Serum total cholesterol (T-CHOL) and triglyceride (TG) contents in the 0.53% group were significantly lower than in all other groups (P<0.05), whereas these values in the 0.80% group were significantly higher than in all other groups (P<0.05). 4) No significant differences were detected in serum superoxide dismutase (SOD) or acid phosphatase (ACP) activities among groups (P>0.05). Serum alkaline phosphatase (AKP) activity in the 2.12% and 2.56% groups was significantly lower than in all other groups except the 1.62% group (P<0.05). Serum catalase (CAT) activity in the 1.62% group was significantly lower than in all other groups except the 1.22% and 2.12% groups (P<0.05). 5) With increasing dietary n-3HUFA levels, liver contents of C18:3n-3, C20:5n-3, and C22:6n-3 increased significantly (P<0.05), while liver C18:2n-6 content decreased significantly (P<0.05). 6) Muscle C18:2n-6 and C20:4n-6 contents showed a decreasing trend with increasing dietary n-3HUFA levels, with the 2.12% and 2.56% groups being significantly lower than all other groups (P<0.05). Muscle C20:5n-3 and C22:6n-3 contents showed an increasing trend, with the 2.56% group being significantly higher than all other groups (P<0.05). In conclusion, based on WGR, broken-line model analysis indicated that the optimal dietary n-3HUFA requirement for juvenile Sillago sihama is 2.21%.

Keywords: Sillago sihama; n-3 highly unsaturated fatty acid; growth performance; serum biochemical indices; tissue fatty acid content

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Sillago sihama belongs to the order Perciformes, suborder Percoidei, family Sillaginidae, and genus Sillago. Commonly known as sand whiting or sand borer, this species is widely distributed in tropical waters of the Indian and Western Pacific Oceans, including the Bohai Sea and South China Sea along China' s coast, with particularly high yields in western Guangdong. As a small carnivorous fish prized for its delicate flavor, Sillago sihama holds significant economic and nutritional value and has historically played an important role in China' s coastal fisheries. However, overfishing in recent years has led to declining wild populations, reduced individual sizes, and depleted natural resources [1].

Highly unsaturated fatty acids (HUFAs) are defined as unsaturated fatty acids containing 20 carbon atoms and 3 double bonds. Based on the position of the first double bond from the methyl end, HUFAs are classified into n-3, n-6, and other series, with n-3HUFA primarily comprising eicosapentaenoic acid (EPA)

and docosahexaenoic acid (DHA). The physiological functions of n-3HUFA include inhibiting pro-inflammatory cytokine production, reducing inflammatory responses and disease symptoms; serving as essential structural components of the retina to promote visual development; and acting as crucial components of cell membranes that influence membrane function and fluidity while playing a major role in intercellular signal transduction [2-4]. In marine fish, EPA and DHA serve as precursors for highly active paracrine hormones such as eicosanoids, participating in physiological regulation [5]. Previous studies have demonstrated that most freshwater fish can synthesize EPA and DHA, requiring only dietary supplementation with linolenic acid as a precursor to meet their n-3HUFA requirements [6]. For marine fish lacking fatty acid elongation and desaturation capacity, n-3HUFA represents an essential fatty acid [6]. Dietary n-3HUFA supplementation can enhance growth performance, maintain lipid metabolism balance, and strengthen immune function in marine fish [7-10].

Since the breakthrough in artificial breeding technology for Sillago sihama in 2012 [11], aquaculture production has expanded rapidly in western Guangdong. However, as an emerging aquaculture species, research on its nutrition and feed formulation remains unreported, severely constraining large-scale cultivation. Therefore, this study investigated the effects of dietary n-3HUFA levels on growth performance, morphological indices, body composition, serum biochemical parameters, enzyme activities, and tissue fatty acid contents of Sillago sihama to determine the optimal dietary n-3HUFA requirement for juveniles and provide fundamental data for developing efficient formulated feeds.

1.1 Experimental Design and Diet Preparation

Six isonitrogenous and isolipidic experimental diets were formulated with graded n-3HUFA levels of 0.53%, 0.80%, 1.22%, 1.62%, 2.12%, and 2.56%. Fish meal and wheat gluten served as protein sources, while fish oil and corn oil were used as lipid sources. The dietary n-3HUFA levels were adjusted by varying the proportions of corn oil and fish oil. Dietary composition and nutrient levels are presented in Table 1 , and fatty acid compositions are shown in Table 2 . Feed ingredients were ground to pass through a 60-mesh sieve and weighed according to formulation. During diet preparation, micro-ingredients were mixed using the progressive enlargement method, followed by addition of varying amounts of fish oil and corn oil. After thorough mixing, 30-40% water was added to form a dough, which was extruded into 1.5 mm pellets using a twin-screw extruder. The pellets were air-dried in a cool, dry environment, sealed in bags, and stored at -20°C.

1.2 Experimental Animals and Management

The feeding trial was conducted at the Marine Biology Research Base of Guangdong Ocean University on Donghai Island, Zhanjiang. Juvenile Sillago sihama were purchased from the Longhaitian Hatchery Experimental Base in Zhanjiang as overwintered fry from the same year. Prior to the experiment, fish were ac-

climated in outdoor cement tanks (4.5 m × 4.9 m × 1.8 m) until reaching the experimental size. At the start of the trial, fish were fasted for 24 hours before distribution. According to the experimental design, 540 healthy fish with uniform size and average weight of (6.0±0.6) g were randomly assigned to six groups with three replicates each. Each replicate consisted of 30 fish stocked in a 0.5 m^3 fiberglass tank. Fish were hand-fed to apparent satiation twice daily (08:00 and 17:00). Mortalities were recorded, weighed, and dissected to determine cause of death. Water quality was monitored regularly. The trial began in June and lasted eight weeks using a recirculating seawater system. During the experimental period, water temperature was maintained at 23-26°C, dissolved oxygen at 5-6 mg/L with continuous aeration, and salinity at 26-28.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection At the end of the feeding trial, fish were fasted for 24 hours before sampling. Fish from each tank were anesthetized with eugenol, weighed, and counted for growth index calculation. Five fish per tank were randomly selected for measurement of body length, body weight, and liver weight to calculate morphological indices. Another five fish were blotted dry, numbered, and stored at -20°C for proximate composition analysis. Additionally, ten fish were sampled for cardiac blood collection. Blood was placed in 1.5 mL centrifuge tubes, stored overnight at -20°C, and then centrifuged at 4,000 r/min for 10 minutes. The serum supernatant was collected and stored at -80°C for biochemical analysis. Livers and muscle tissues were excised, snap-frozen in liquid nitrogen, and transferred to -80°C storage for subsequent enzyme activity and fatty acid composition analyses.

1.3.2 Proximate Composition Analysis Proximate composition of whole fish and diets was analyzed using standard international methods [12]. Moisture content was determined by oven-drying at 105°C. Crude protein content was measured using a KjeltecTM 8400 analyzer (Sweden). Crude lipid content was determined by Soxhlet extraction with petroleum ether. Crude ash content was measured by incineration in a muffle furnace at 550°C.

1.3.3 Fatty Acid Analysis Fatty acid composition was determined according to GB/T 21514–2008. The methodology involves saponification with sodium hydroxide-methanol solution, followed by conversion to fatty acid methyl esters via reaction with boron trifluoride-methanol solution. Separation was performed by capillary gas chromatography, with peaks identified using standard reference materials of known composition and quantified by internal standard calibration.

1.3.4 Serum Biochemical Indices Serum triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (T-CHOL) contents, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, were measured using

an automatic biochemical analyzer (Hitachi 7600-110) with commercial kits purchased from Nanjing Weitman Biological Technology Co., Ltd.

1.3.5 Tissue Enzyme Activity Assays Activities of catalase (CAT), superoxide dismutase (SOD), alkaline phosphatase (AKP), and acid phosphatase (ACP) in liver tissue were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s protocols.

1.4 Calculation Formulas

Weight gain rate (WGR, %) = [(final mean weight - initial mean weight) / initial mean weight] × 100
Specific growth rate (SGR, %/d) = [(ln final mean weight - ln initial mean weight) / feeding days] × 100
Feed conversion ratio (FCR) = dry feed intake / (final mean weight - initial mean weight)
Survival rate (SR, %) = (final fish number / initial fish number) × 100
Hepatosomatic index (HSI, %) = (liver weight / body weight) × 100
Condition factor (CF, g/cm^3) = [final weight (g) / body length^3 (cm)] × 100

1.5 Statistical Analysis

Data are expressed as means ± standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS 20.0. When significant differences were detected, Duncan' s multiple range test was applied. Statistical significance was set at $P < 0.05$.

2.1 Effects of Dietary n-3HUFA Level on Growth Performance and Morphological Indices

As shown in Table 3 , WGR increased initially and then decreased with increasing dietary n-3HUFA levels, reaching its maximum in the 2.12% group, which was significantly higher than the 0.53% and 0.80% groups ($P<0.05$) but not significantly different from other groups ($P>0.05$). SGR followed a similar trend, with the highest value in the 2.12% group, significantly higher than the 0.53% and 2.56% groups ($P<0.05$) but not significantly different from other groups ($P>0.05$). FCR decreased initially and then increased, with the lowest value in the 2.12% group, significantly lower than the 0.53% group ($P<0.05$) but not significantly different from other groups ($P>0.05$). No significant differences were observed in SR among groups ($P>0.05$). HSI decreased initially and then plateaued, reaching its minimum in the 2.12% group, which was significantly lower than the 0.53% and 0.80% groups ($P<0.05$) but not significantly different from other groups ($P>0.05$). CF increased initially and then decreased, peaking in the 2.12% group, which was significantly higher than all groups except the 1.62% group ($P<0.05$).

As illustrated in Figure 1 [Figure 1: see original paper], broken-line model analysis based on WGR indicated that the optimal dietary n-3HUFA requirement for Sillago sihama is 2.21%.

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

As presented in Table 4 , no significant differences were detected in whole-body moisture, crude protein, or crude ash content among groups ($P>0.05$). Whole-body crude lipid content increased initially and then decreased with rising dietary n-3HUFA levels. No significant difference was observed between the 1.62% and 2.12% groups ($P>0.05$), but both were significantly higher than all other groups ($P<0.05$), among which no significant differences existed ($P>0.05$).

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

Table 5 shows that serum HDL-C content increased significantly when dietary HUFA levels rose from 0.53% to 1.62% ($P<0.05$), with the 2.56% group exhibiting significantly higher values than all groups except the 2.12% group ($P<0.05$). Serum LDL-C content increased initially and then decreased gradually, reaching its maximum in the 0.80% group, which was significantly higher than all other groups ($P<0.05$). Serum ALT and AST activities displayed a pattern of initial decrease, followed by increase, then decrease again. ALT activity reached its minimum in the 1.22% group, significantly lower than all other groups ($P<0.05$), while AST activity was lowest in the 0.80% group, significantly lower than all other groups ($P<0.05$). Serum T-CHOL content increased initially and then decreased and stabilized, whereas TG content increased initially and then decreased. The 0.53% group showed significantly lower T-CHOL and TG contents than all other groups ($P<0.05$), while the 0.80% group had significantly higher values than all other groups ($P<0.05$).

2.4 Effects of Dietary n-3HUFA Level on Liver Enzyme Activities

As shown in Table 6 , no significant differences were observed in serum SOD or ACP activities among groups ($P>0.05$). Serum AKP activity did not differ significantly between the 2.12% and 2.56% groups ($P>0.05$) but was significantly lower than all other groups except the 1.62% group ($P<0.05$). Serum CAT activity was lowest in the 1.62% group, not significantly different from the 1.22% and 2.12% groups ($P>0.05$) but significantly lower than all other groups ($P<0.05$).

2.5 Effects of Dietary n-3HUFA Level on Liver and Muscle Fatty Acid Composition

Table 7 reveals that the predominant fatty acids in Sillago sihama liver were C16:0 and C18:1n-9, each exceeding 20% of total fatty acids. With increas-

ing dietary n-3HUFA levels, liver contents of C18:3n-3, C20:5n-3, and C22:6n-3 increased significantly ($P<0.05$), while liver C18:2n-6 content decreased significantly ($P<0.05$). Liver C18:1n-9 content showed a decreasing trend, whereas C18:0 content increased initially and then stabilized, peaking in the 2.12% group.

Table 8 demonstrates that muscle C18:2n-6 and C20:4n-6 contents decreased with rising dietary n-3HUFA levels, with the 2.12% and 2.56% groups being significantly lower than all other groups ($P<0.05$). Muscle C20:5n-3 and C22:6n-3 contents increased progressively, with the 2.56% group significantly higher than all other groups ($P<0.05$). Muscle C16:0 content increased initially and then stabilized, reaching its maximum in the 1.22% group, while C16:1n-9 content peaked in the 1.62% group.

3.1 Effects of Dietary n-3HUFA Level on Growth Performance and Morphological Indices

Previous studies have demonstrated that optimal dietary n-3HUFA levels can promote growth and improve feed utilization in marine fish [13], though requirements vary considerably among species. Black seabream (Sparus macrocephalus) juveniles achieved maximum WGR at 0.85% dietary n-3HUFA [14], while Brachymystax lenok showed significantly improved WGR and SGR at 0.75% n-3HUFA [15]. Kim et al. [16] recommended dietary n-3HUFA levels of 0.8-1.0% for juvenile flounder (Paralichthys olivaceus). Large yellow croaker (Larmichthys crocea) exhibited significantly enhanced growth performance at 0.98% n-3HUFA [17], and gilthead seabream (Sparus aurata) juveniles showed optimal growth and feed efficiency at 1% n-3HUFA [18]. Orange-spotted grouper (Epinephlus coioides) juveniles and sub-adults achieved maximum SGR at 2.53% and 3.93% n-3HUFA, respectively [19]. The present results indicate that dietary n-3HUFA level significantly affected WGR, SGR, and FCR in Sillago sihama, following a trend of initial improvement followed by stabilization. Broken-line model analysis based on WGR suggested maximum growth at 2.21% dietary n-3HUFA.

In contrast, studies on rabbitfish (Siganus canaliculatus) [20] and Russian sturgeon (Acipenser gueldenstaedti) juveniles [21] found no significant effects of dietary n-3HUFA on WGR, SGR, or FCR, with HSI showing an initial increase followed by decrease. These discrepancies may relate to species-specific feeding habits. Herbivorous fish consume diets richer in linoleic acid than linolenic acid, potentially creating a dietary requirement for linoleic acid. As a herbivorous species, rabbitfish showed minimal or no response to varying n-3HUFA levels. Species-specific differences in fatty acid metabolism also influence results; Atlantic salmon (Salmo salar) can synthesize EPA and DHA from 18:2n-6, with synthesis capacity affected by environmental factors [22]. In this study, HSI in Sillago sihama decreased initially and then increased with rising dietary n-3HUFA, consistent with findings in gilthead seabream [18] and orange-spotted grouper juveniles [23] but contrary to results in rabbitfish [20]. Nutritional defi-

ciencies or excesses can cause morphological changes in cultured organisms [18]. Research on orange-spotted grouper indicated that n-3HUFA can prevent hepatic stellate cell production and reduce HSI [19], similar to our results, suggesting that dietary n-3HUFA promotes lipid catabolism and reduces fat deposition, particularly in the liver [24-25]. CF is an important indicator of fish body conformation. The initial increase followed by decrease in CF with rising dietary n-3HUFA suggests that moderate levels benefit morphological development in Sillago sihama, consistent with results in orange-spotted grouper juveniles [19] but differing from studies on orange-spotted grouper [23], cobia (Rachycentron canadum L.) [26], and grass carp (Ctenopharyngodon idella) larvae [27] that reported no significant effects on CF.

3.2 Effects of Dietary n-3HUFA Level on Body Composition

In this study, dietary n-3HUFA level did not significantly affect moisture, crude protein, or crude ash content in Sillago sihama, similar to findings in cobia [28] and Brachymystax lenok [15]. Whole-body crude lipid content increased initially and then decreased with rising dietary n-3HUFA, consistent with results in cobia [26], flounder [29], and grass carp larvae [27]. This pattern may occur because excess n-3HUFA beyond metabolic requirements is deposited in tissues. Once lipid deposition requirements are met, n-3HUFA may induce uncoupling proteins (UCP) in mitochondria, enhancing -oxidation and reducing lipid deposition [2,30]. Conversely, studies on orange-spotted grouper [19], black seabream [31], and Pacific white shrimp (Litopenaeus vannamei) [32] reported opposite trends, with body lipid decreasing initially and then increasing with dietary n-3HUFA. Other studies on turbot [33], rabbitfish [20], and red seabream (Pagrus major) [34] found no significant effects of dietary n-3HUFA on body crude lipid content. These variations indicate that effects of dietary n-3HUFA on body composition are species-specific.

3.3 Effects of Dietary n-3HUFA Level on Serum Biochemical Indices

Serum biochemical parameters serve as indicators of organismal health status, providing insights into nutrition, metabolism, and disease conditions. Liu et al. [35] reported that DHA effectively reduced serum T-CHOL and TG in hyperlipidemic patients. EPA and DHA significantly decreased serum T-CHOL in laying hens [36] and inhibited T-CHOL and TG synthesis, reducing their serum concentrations in humans [37]. EPA and DHA also improve blood circulation and reduce low-density lipoprotein levels that can induce arteriosclerosis and thrombosis [38]. In this study, serum HDL-C content increased gradually while T-CHOL increased initially and then decreased, similar to results in Russian sturgeon juveniles [21]. Serum ALT and AST activities decreased initially and then increased, possibly because elevated dietary n-3HUFA increased hepatic lipid metabolism burden, reducing cell membrane permeability and limiting ALT and AST release into blood [26]. HDL-C protects cardiovascular function

and promotes reverse cholesterol transport [39], whereas LDL-C is the primary vehicle for transporting endogenous cholesterol from liver to tissues [40]. The transient increase followed by continuous decrease in serum LDL-C with rising dietary n-3HUFA in Sillago sihama resembles findings in Pacific white shrimp [32], indicating that n-3HUFA benefits lipid metabolism.

3.4 Effects of Dietary n-3HUFA Level on Liver Enzyme Activities

In organisms, SOD, CAT, and peroxidase (POD) constitute the reactive oxygen species defense system, effectively scavenging superoxide radicals, hydrogen peroxide, and other peroxides [41]. CAT participates in reactive oxygen metabolism by catalyzing hydrogen peroxide decomposition, maintaining free radical balance and preventing peroxidative damage [42]. AKP, a non-specific phosphomonoesterase, catalyzes hydrolysis of all phosphomonoesters to produce phosphate ions and free hydroxyl groups [43] and participates in hepatocellular excretory function when present in liver [44]. In this study, dietary n-3HUFA did not affect liver SOD or ACP activities. However, liver CAT activity decreased gradually as dietary n-3HUFA increased from 0.80% to 1.62%, then increased as levels rose from 1.62% to 2.56%, similar to results in orange-spotted grouper [19]. This suggests that dietary n-3HUFA levels below 1.62% benefit reactive oxygen metabolism and superoxide radical balance, while levels above 1.62% induce increased CAT activity to scavenge free radicals generated from high-level n-3HUFA peroxidation. In contrast, studies on marbled rockfish (Sebastiscus marmoratus) [45] reported no effect of dietary n-3HUFA on liver CAT activity.

3.5 Effects of Dietary n-3HUFA Level on Liver and Muscle Fatty Acid Composition

Dietary n-3HUFA level can significantly affect tissue fatty acid composition [46-50], which partially reflects dietary fatty acid profiles [51]. In this study, liver n-3HUFA content increased with dietary n-3HUFA level, consistent with dietary patterns, similar to findings in Pacific white shrimp [32], black seabream juveniles [52], and red seabream [34]. In marine fish, C18:1n-9 content can indicate essential fatty acid deficiency [19]. The marked decrease in liver C18:1n-9 with increasing dietary n-3HUFA suggests that dietary supplementation progressively meets the essential fatty acid requirement of Sillago sihama. The more pronounced effect of dietary n-3HUFA on liver fatty acid composition compared to muscle resembles results in flounder [8]. Research indicates that animal tissues provide ATP through -oxidation of fatty acids according to the hierarchical preference of n-9 > n-6 > n-3 [53]. The significant reduction in liver C18:1n-9 suggests Sillago sihama can utilize monounsaturated fatty acids for energy metabolism, as reported in orange-spotted grouper [19] and red seabream [54]. Higher EPA and DHA contents in muscle compared to dietary levels indicate selective retention of these fatty acids in vivo, consistent with studies on Pacific white shrimp [55] and tiger prawn [56]. The n-3/n-6 ratio is commonly

used to evaluate fatty acid nutritional value, with higher ratios indicating better nutritional quality [57]. Lipids affect muscle flavor and texture, and HUFA degradation during heating generates characteristic aromatic aldehydes and ketones [58], demonstrating that dietary n-3HUFA can improve flesh quality in Sillago sihama.

4 Conclusion

Under the conditions of this experiment, comprehensive evaluation of all measured parameters indicates that dietary n-3HUFA supplementation at 1.62-2.12% promotes growth performance and maintains internal homeostasis in Sillago sihama. Based on weight gain rate, broken-line model analysis determined the optimal dietary n-3HUFA requirement for juvenile Sillago sihama to be 2.21%.

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