Effects of Dietary Linolenic Acid Content on Growth Performance, Antioxidant Indices, and Serum Biochemical Parameters in Large-Size Japanese Seabass (Postprint)

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Abstract

This study aimed to investigate the effects of dietary alpha-linolenic acid (ALA) content on growth performance and serum biochemical indices of large-size largemouth bass, in order to determine the optimal dietary ALA level for large-size largemouth bass. Six iso-nitrogenous and iso-lipidic experimental diets with ALA contents of 0.06%, 0.99%, 2.03%, 3.18%, 4.12%, and 5.08% of dry diet weight were prepared by adding perilla oil to the basal diet. The above experimental diets were fed to largemouth bass with an initial body weight of (207.77$±$0.64) g, with 3 replicates per diet and 20 fish per replicate, for a culture period of 12 weeks. The results showed that: 1) Specific growth rate (SGR) and feed efficiency (FE) both exhibited a trend of increasing first and then decreasing with increasing dietary ALA content, with maximum values observed in the 2.03% ALA group, while no significant differences in SGR and FE were found between the 2.03% and 3.18% ALA groups (P>0.05). Hepatosomatic index (HSI) and viscerosomatic index (VSI) reached maximum values in the 5.08% ALA group, which were significantly higher than those in the 0.06% ALA group (P<0.05). Survival rate (SR) and condition factor (CF) showed no significant differences among all groups (P>0.05). 2) With increasing dietary ALA content, whole-body crude protein content showed a gradual decreasing trend, while crude lipid content exhibited an increasing trend. Whole-body crude protein content in the 4.12% and 5.08% ALA groups was significantly lower than that in the 0.06% ALA group (P<0.05), while whole-body crude lipid content in the 4.12% and 5.08% ALA groups was significantly higher than that in the 0.06%, 0.99%, 2.03%, and 3.18% ALA groups (P<0.05). No significant differences in moisture and crude ash contents were observed among different groups (P>0.05). 3) Serum and liver superoxide dismutase (SOD) ac-

tivities in the 2.03% ALA group showed no significant differences compared with the 3.18% ALA group (P>0.05), but were significantly higher than those in the 0.06% and 5.08% ALA groups (P<0.05). Serum MDA content in the 2.03% and 3.18% ALA groups was significantly lower than that in the 0.06% and 5.08% ALA groups (P<0.05); meanwhile, liver MDA content reached the minimum in the 2.03% ALA group, which was not significantly different from the 3.18% ALA group (P>0.05) but significantly lower than all other groups (P<0.05). 4) Serum aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities were both lowest in the 2.03% ALA group, which were significantly lower than those in the 0.06% and 5.08% ALA groups (P<0.05). Serum triglyceride (TG) and cholesterol (CHOL) contents reached maximum values in the 4.12% ALA group, which were significantly higher than those in the 0.06% ALA group (P<0.05). With increasing dietary ALA content, serum high-density lipoprotein cholesterol (HDL-C) content exhibited a trend of increasing first and then decreasing, with the 2.03%, 3.18%, and 4.12% ALA groups being significantly higher than other groups (P<0.05). In summary, appropriate dietary ALA content (2.03%~3.18%) could promote growth, enhance antioxidant capacity, and improve liver health status in large-size largemouth bass; using SGR and FE as evaluation indicators, quadratic curve regression analysis determined that the optimal dietary ALA contents for largemouth bass with body weights of 207.77~406.94 g were 2.53% and 2.72% of dry diet weight, respectively.

Full Text

Effects of Dietary α-Linolenic Acid Content on Growth Performance, Antioxidant Indices and Serum Biochemical Indices of Large Size Japanese Seabass (Lateolabrax japonicus)

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Abstract

A 12-week feeding experiment was conducted to investigate the effects of dietary α-linolenic acid (ALA) content on growth performance, antioxidant indices, and serum biochemical indices of large size Japanese seabass (Lateolabrax japonicus) to determine the optimal dietary ALA requirement. Six isonitrogenous and isolipidic experimental diets were formulated by supplementing a basal diet with perilla oil to achieve dietary ALA levels of 0.06%, 0.99%, 2.03%, 3.18%,

4.12%, and 5.08% of dry weight. Japanese seabass with an initial body weight of (207.77$±$0.64) g were fed the experimental diets, with three replicates per diet and 20 fish per replicate. The results showed: 1) Specific growth rate (SGR) and feed efficiency (FE) initially increased and then decreased with rising dietary ALA content, reaching maximum values in the 2.03% ALA group, with no significant differences between the 2.03% and 3.18% ALA groups (P>0.05). Hepatosomatic index (HSI) and viscerasomatic index (VSI) peaked in the 5.08% ALA group, significantly higher than in the 0.06% ALA group (P<0.05). Survival rate (SR) and condition factor (CF) did not differ significantly among groups (P>0.05). 2) Whole-body crude protein content gradually decreased with increasing dietary ALA, while crude lipid content increased. The 4.12% and 5.08% ALA groups showed significantly lower crude protein content than the 0.06% ALA group (P<0.05), and significantly higher crude lipid content than the 0.06%, 0.99%, 2.03%, and 3.18% ALA groups (P<0.05). No significant differences were observed in moisture or ash content among groups (P>0.05). 3) Serum and liver superoxide dismutase (SOD) activity in the 2.03% ALA group did not differ significantly from the 3.18% ALA group (P>0.05) but was significantly higher than in the 0.06% and 5.08% ALA groups (P<0.05). Serum malondialdehyde (MDA) content in the 2.03% and 3.18% ALA groups was significantly lower than in the 0.06% and 5.08% ALA groups (P<0.05), while liver MDA content reached its minimum in the 2.03% ALA group, significantly lower than all other groups except the 3.18% ALA group (P<0.05). 4) Serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities were lowest in the 2.03% ALA group, significantly lower than in the 0.06% and 5.08% ALA groups (P<0.05). Serum triglyceride (TG) and cholesterol (CHOL) contents peaked in the 4.12% ALA group, significantly higher than in the 0.06% ALA group (P<0.05). Serum high-density lipoprotein cholesterol (HDL-C) content initially increased and then decreased with rising dietary ALA, with the 2.03%, 3.18%, and 4.12% ALA groups showing significantly higher values than other groups (P<0.05). In conclusion, optimal dietary ALA content (2.03%~3.18%) promoted growth, enhanced antioxidant capacity, and improved liver health in large size Japanese seabass. Based on quadratic regression analysis using SGR and FE as evaluation criteria, the optimal dietary ALA contents for Japanese seabass weighing 207.77~406.94 g were determined to be 2.53% and 2.72% of dry weight, respectively.

Keywords: large size Japanese seabass; α-linolenic acid; growth performance; serum biochemical indices; antioxidant ability

Introduction

α-Linolenic acid (ALA) is an n-3 polyunsaturated fatty acid primarily derived from vegetable oils such as perilla oil and linseed oil, playing important roles in promoting animal growth, enhancing immunity, improving meat quality, and reducing blood lipids [1-2]. Previous studies have demonstrated that some fresh-

water fish can convert ALA into essential long-chain n-3 polyunsaturated fatty acids through desaturation and carbon chain elongation to meet their physiological requirements [3-4]. While marine and euryhaline fish possess relatively weaker conversion capacity, research has confirmed that substituting a proportion of fish oil with ALA-rich vegetable oils does not affect growth and survival in some marine and euryhaline species, and may even provide certain benefits [5-6].

Research on ALA requirements in fish has primarily focused on freshwater species. Jiang et al. [7] reported that dietary ALA at 0.5% significantly improved specific growth rate and feed efficiency in crucian carp. Bogut et al. [8] documented a 1% ALA requirement for European catfish. Guan [9] found that 1.2% dietary ALA produced optimal growth in tilapia. For marine and euryhaline fish, whose essential fatty acids are highly unsaturated fatty acids [arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)], numerous studies have investigated requirements for these highly unsaturated fatty acids [10-11]. However, research on ALA requirements remains limited, with most experiments focusing on fish oil substitution rather than establishing quantitative requirements. Serum biochemical indices serve as important indicators of organismal health, and recent studies on turbot [10], common carp [12], and Murray cod [13] have confirmed that varying dietary ALA levels affect serum biochemical parameters, consequently impacting organismal and hepatic health.

Japanese seabass (Lateolabrax japonicus) is a euryhaline fish belonging to the family Serranidae, order Perciformes. Known for its rapid growth and high economic value, it represents an important aquaculture species in China' s coastal regions. Although numerous studies have investigated fatty acid nutrition in Japanese seabass, research on the effects of ALA on growth, antioxidant capacity, and liver health in large size individuals remains scarce. Therefore, this study examined the effects of dietary ALA content on growth performance, antioxidant indices, and serum biochemical indices in large size Japanese seabass to determine optimal dietary ALA requirements, providing a theoretical foundation for ALA nutrition in euryhaline fish and contributing to the nutritional database for Japanese seabass.

Materials and Methods

1.1 Experimental Diets A basal diet was formulated using fish meal, soybean meal, and casein as primary protein sources, and wheat flour as the main carbohydrate source, containing approximately 44% crude protein and 12% crude lipid. Perilla oil (Jiangsu TianKai Biological Technology Co., Ltd.; major fatty acid composition: C16:0, 5.96%; C18:0, 2.63%; C18:1n-9, 21.18%; C18:2n-6, 14.61%; C18:3n-3, 54.85%) was added at 0%, 2%, 4%, 6%, 8%, and 10% to create six isonitrogenous and isolipidic experimental diets. Tristearin (Jiangsu TianKai Biological Technology Co., Ltd.; major fatty acid composition: C14:0, 2.56%; C16:0, 27.74%; C18:0, 64.27%) was used to balance lipid

content. Gas chromatography analysis confirmed dietary ALA levels of 0.06% (control), 0.99%, 2.03%, 3.18%, 4.12%, and 5.08% of dry weight, designated as groups A1 through A6, respectively. Dietary composition and nutrient levels are presented in Table 1 , and fatty acid composition is shown in Table 2 . All feed ingredients were ground and mixed quantitatively in ascending order of proportion. Perilla oil was thoroughly blended with the dry ingredients, water was added for pelleting, and the pellets were dried at approximately 50 °C and stored in a cool, dry place.

1.2 Experimental Fish and Culture Management The feeding trial was conducted at Xiangshan Bay Seed Co., Ltd., Zhejiang Province, using marine floating cages over a 12-week period. Experimental fish were one-year-old artificially propagated Japanese seabass obtained from a farmer in Xiangshan County, Ningbo. All fish were initially stocked in large net cages (3.0 m × 3.0 m × 3.0 m) and acclimated to the experimental diets and culture environment for 15 days using the control diet. Prior to the experiment, fish were fasted for 24 h and anesthetized with eugenol (1:10,000) for weighing. Uniform-sized Japanese seabass [initial body weight (207.77$±$0.64) g] were randomly distributed into 18 culture net cages (1.5 m × 1.5 m × 2.0 m) at 20 fish per cage. Each experimental diet was randomly assigned to three replicate cages. Fish were hand-fed twice daily (06:00 and 17:00) to apparent satiation. During the experimental period, water temperature ranged from 23.0 to 30.5 °C, salinity from 26‰ to 31‰, and dissolved oxygen concentration was approximately 6.5 mg/L.

At the end of the 12-week trial, fish were fasted for 24 h, counted, and weighed. Three fish were randomly selected from each cage, sealed in bags, and stored at -20 °C for whole-body proximate composition analysis. Additionally, five fish per cage were randomly sampled for tissue collection (liver, muscle, intestine), which were immediately frozen in liquid nitrogen and stored at -80 °C. Blood (approximately 1.5 mL) was collected from the caudal vein of four fish per cage, allowed to clot at 4 °C for 4 h, centrifuged at 3,000 r/min for 10 min, and the serum was harvested and stored at -80 °C.

1.4.1 Growth Performance Growth performance was calculated using the following formulas: Survival rate (SR, %) = 100 × (final fish number / initial fish number); Weight gain rate (WGR, %) = 100 × (final body weight - initial body weight) / initial body weight; Specific growth rate (SGR, %/d) = 100 × [ln(final body weight) - ln(initial body weight)] / experimental days; Feed efficiency (FE) = (final body weight - initial body weight) / dry weight of feed consumed; Hepatosomatic index (HSI, %) = 100 × (wet liver weight / body weight); Viscerasomatic index (VSI, %) = 100 × (wet viscera weight / body weight); Condition factor (CF, %) = 100 × body weight / body length^3 (body weight in g; body length in cm).

1.4.2 Antioxidant Indices and Serum Biochemical Indices Serum and liver malondialdehyde (MDA) content and superoxide dismutase (SOD) activity

were measured using commercial assay kits from Nanjing Jiancheng Bioengineering Institute. Serum biochemical indices were determined using an automatic biochemical analyzer (BS-200, Mindray Medical International Limited) with corresponding reagent kits, including glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase (AKP) activities, and triglycerides (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents.

1.4.3 Proximate Composition of Fish and Tissues Moisture and ash contents were determined by weight loss method. Crude protein content was measured by the Kjeldahl method using a VELP UDK-142 automatic Kjeldahl analyzer (Italy). Crude lipid content was determined by the Soxhlet extraction method using a FOSS SOXTEC-2050 Soxhlet extractor (Sweden).

1.4.4 Dietary Fatty Acid Composition Dietary fatty acid composition was analyzed by gas chromatography following Mourente et al. [14] with slight modifications. Briefly, 100 mg of freeze-dried, ground sample was placed in a 15 mL headspace vial, mixed with 3 mL of 1 mol/L KOH-methanol solution, and heated at 75 °C for 20 min. After cooling to room temperature, 3 mL of 2 mol/L HCl-methanol solution was added and heated at 75 °C for 20 min. Following cooling, 1.5 mL of n-hexane (chromatographic grade) was added for extraction. The upper hexane layer containing fatty acid methyl esters was carefully collected, and 1 L was injected into a gas chromatograph (HP5890II, USA) with flame ionization detection. Fatty acids were identified by comparing retention times with standards, and relative proportions were determined by peak area normalization.

1.5 Data Processing and Analysis Results are expressed as mean ± standard error (mean±SE). Data were analyzed using SPSS 19.0 software. One-way analysis of variance (one-way ANOVA) was performed, and when significant differences were detected, Tukey' s test was used for multiple comparisons. Significance was accepted at $P<0.05$.

Results

2.1 Effects of Dietary ALA Content on Growth Performance of Large Size Japanese Seabass As shown in Table 3 , survival rates across all groups ranged from 95.00% to 100.00% with no significant differences ($P>0.05$). Weight gain rate (WGR) and specific growth rate (SGR) initially increased and then decreased with rising dietary ALA content. The A3 group exhibited significantly higher WGR and SGR than the A1, A2, and A6 groups ($P<0.05$), with no significant differences from the A4 and A5 groups ($P>0.05$). Feed efficiency (FE) significantly increased with dietary ALA content from 0.06% to 2.03% ($P<0.05$), then declined, reaching its maximum in the A3 group. Hepatosomatic index (HSI) and viscerasomatic index (VSI) were significantly affected by dietary

ALA content ($P<0.05$), peaking in the A6 group and significantly higher than in the A1 group ($P<0.05$). Condition factor (CF) did not differ significantly among groups ($P>0.05$).

Quadratic regression analysis (Figure 1 [Figure 1: see original paper]) with dietary ALA content (x) as the independent variable and SGR (y) as the dependent variable yielded the equation $y = -0.0198x^2 + 0.1001x + 0.7302$ ($R^2 = 0.8929$). The regression indicated maximum SGR at 2.53% dietary ALA content. Similarly, quadratic regression analysis (Figure 2 [Figure 2: see original paper]) of dietary ALA content (x) versus FE (y) produced the equation $y = -0.0263x^2 + 0.1432x + 0.4739$ ($R^2 = 0.9483$), indicating maximum FE at 2.72% dietary ALA content.

2.2 Effects of Dietary ALA Content on Proximate Composition of Whole Body and Tissues As shown in Table 4 , whole-body crude protein content gradually decreased with increasing dietary ALA, with the A5 and A6 groups significantly lower than the A1 group ($P<0.05$). Whole-body crude lipid content increased with dietary ALA, with the A5 and A6 groups significantly higher than the A1, A2, A3, and A4 groups ($P<0.05$). No significant differences were observed in moisture or ash content among groups ($P>0.05$). Liver crude lipid content in the A3 group was significantly lower than in the A1 and A2 groups ($P<0.05$), with no other significant differences among groups. Muscle crude lipid content initially decreased and then increased with dietary ALA, with the A4 group significantly higher than the A1 and A2 groups ($P<0.05$).

2.3 Effects of Dietary ALA Content on Antioxidant Indices As shown in Table 5 , serum SOD activity initially increased and then decreased with rising dietary ALA, peaking in the A3 group. This value did not differ significantly from the A4 group ($P>0.05$) but was significantly higher than the other four groups ($P<0.05$). Liver SOD activity followed the same trend. Serum MDA content decreased initially and then increased with dietary ALA, with the A3 and A4 groups significantly lower than the A1 and A6 groups ($P<0.05$). Liver MDA content reached its minimum in the A3 group, significantly lower than all other groups except the A4 group ($P<0.05$).

2.4 Effects of Dietary ALA Content on Serum Biochemical Indices As shown in Table 6 , serum GOT and GPT activities initially decreased and then increased with rising dietary ALA. The A3 and A4 groups exhibited significantly lower GOT activity than other groups ($P<0.05$), while the A3, A4, and A5 groups showed significantly lower GPT activity than other groups ($P<0.05$). Serum AKP activity did not differ significantly among groups ($P>0.05$). Serum CHOL and TG contents significantly increased from 4.76 nmol/L to 6.28 nmol/L and from 3.71 nmol/L to 4.86 nmol/L, respectively, as dietary ALA increased from 0.06% to 4.12% ($P<0.05$). When ALA content further increased to 5.08%, serum CHOL and TG contents significantly decreased to 4.79 nmol/L and 3.99 nmol/L, respectively ($P<0.05$). Serum HDL-C content initially increased and

then decreased with dietary ALA, with the A3, A4, and A5 groups not differing significantly from each other ($P>0.05$) but all significantly higher than other groups ($P<0.05$). Serum LDL-C content did not differ significantly among groups ($P>0.05$).

Discussion

3.1 Effects of Dietary ALA Content on Growth Performance The present results demonstrate that dietary ALA content significantly affected SGR and FE in large size Japanese seabass, with both excessive and deficient ALA levels inhibiting growth and reducing feed utilization. This indicates that optimal dietary ALA content is crucial for growth performance and health in this species. Similar findings have been reported in other marine and euryhaline fish. For instance, Mourente et al. [5] reported good growth in European sea bass fed diets containing 10.6% linseed oil. Xu et al. [15] found optimal growth in juvenile Japanese seabass fed 3% linseed oil. Peng et al. [10] demonstrated that complete replacement of fish oil with linseed oil did not impair growth in juvenile turbot. Friesen et al. [16] observed no significant growth reduction in sablefish fed 10% cold-pressed flaxseed oil. Conversely, Bell et al. [17] reported growth inhibition in juvenile turbot fed 19% linseed oil, and Tu et al. [18] found reduced growth in barramundi fed 14% ALA-rich vegetable oil. These findings suggest that appropriate dietary ALA levels can promote growth in some marine and euryhaline species, though requirements vary among species, fish sizes, and culture conditions.

3.2 Effects of Dietary ALA Content on Antioxidant Capacity Superoxide dismutase (SOD) catalyzes the conversion of superoxide anions to hydrogen peroxide, playing a key role in protecting organisms from oxidative damage by scavenging O_2^- [19]. In this study, serum and liver SOD activities initially increased and then decreased with dietary ALA, with significantly higher values in the A3 and A4 groups compared to the A1 and A6 groups ($P<0.05$). This suggests that optimal dietary ALA enhances antioxidant capacity, consistent with findings by Kiron et al. [20] and Guan [9]. Pan et al. [12] reported that flaxseed oil was comparable to fish oil in enhancing antioxidant capacity. However, excessive ALA reduced SOD activity, potentially impairing immune response, which aligns with results from Montero et al. [21] in gilthead sea bream and Xu et al. [15] in juvenile Japanese seabass. This may be attributed to excessive hepatic lipid deposition at high ALA levels, compromising liver health.

Malondialdehyde (MDA) is a final decomposition product of lipid peroxidation induced by free radicals, with higher levels indicating greater cellular damage. Serum and liver MDA contents showed opposite trends to SOD activity, with significantly higher MDA in the A6 group compared to the A3 and A4 groups. This likely resulted from excessive oxidative stress induced by high ALA content, affecting immune function and elevating MDA levels, similar to findings in

tilapia by Guan [9]. The growth performance results indicated an optimal ALA requirement of 2.53%~2.72%, corresponding to higher SOD activity and lower MDA content, indicating superior antioxidant capacity.

3.3 Effects of Dietary ALA Content on Proximate Composition Numerous studies have shown that dietary composition affects fish proximate composition [22-23]. The present study found that increasing dietary ALA elevated whole-body crude lipid content while reducing crude protein content, suggesting that high dietary ALA promotes lipid deposition in large size Japanese seabass. This may be attributed to differential effects of dietary ALA on the expression of genes related to hepatic fatty acid synthesis and oxidation. These results are consistent with studies in Atlantic salmon [24], GIFT tilapia [25], juvenile Japanese seabass [15], and tilapia [9]. However, other studies found no effect of ALA on proximate composition in trout [26], halibut [27], or turbot [28]. These discrepancies may be related to differences in dietary ALA levels, fish species and sizes, and basal diet formulations.

3.4 Effects of Dietary ALA Content on Serum Biochemical Indices Serum GPT and GOT activities are commonly used enzymatic indicators of liver health [29-30]. These transaminases play important roles in metabolism, and when liver function is compromised, they are released into the bloodstream in large quantities, causing marked increases in serum activities [31]. The present results showed that both low and high dietary ALA levels produced significantly higher serum GPT and GOT activities compared to the optimal A3 and A4 groups, indicating that suboptimal ALA levels adversely affect liver health and consequently growth performance. This is consistent with findings in turbot [10] and Murray cod [13].

Serum TG, CHOL, HDL-C, and LDL-C contents are clinical indicators of lipid metabolism [32-33]. High-density lipoprotein transports cholesterol back to the liver as HDL-C, making serum HDL-C content negatively correlated with cardiovascular disease risk, whereas low-density lipoprotein transports cholesterol from liver to blood as LDL-C. In this study, serum TG and CHOL contents significantly increased as dietary ALA rose from 0.06% to 4.12%, then decreased at 5.08% ALA. This suggests that within a certain range, serum TG and CHOL increase with dietary ALA, consistent with findings in GIFT tilapia [9]. However, the reduction in TG and CHOL at high ALA levels suggests that flaxseed oil may have lipid-lowering effects [34], or alternatively, that high ALA content impaired liver function and disrupted lipid metabolism, reducing serum TG and CHOL levels. Other serum biochemical indices support the latter interpretation, indicating negative impacts of excessive ALA on liver health. Serum HDL-C content initially increased and then decreased with dietary ALA, peaking at 2.03%~4.12% ALA and significantly exceeding values in the A1 and A6 groups. This suggests that optimal dietary ALA benefits liver and cardiovascular health, consistent with results in blunt snout bream [35].

Under the conditions of this study, optimal dietary ALA content (2.03%~3.18%) promoted growth and enhanced antioxidant capacity and liver health in large size Japanese seabass. Quadratic regression analysis based on SGR and FE indicated that the optimal dietary ALA contents for Japanese seabass weighing 207.77~406.94 g were 2.53% and 2.72% of dry weight, respectively.

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