# Effects of Dietary Arachidonic Acid Level on Growth Performance, Antioxidant Capacity, Serum Biochemical Indices, and Liver and Muscle Fatty Acid Composition in Juvenile Pearl Gentian Grouper (Postprint)

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## Abstract

This study aimed to investigate the effects of dietary arachidonic acid (ARA) levels on growth performance, antioxidant capacity, serum biochemical indices, and fatty acid composition in liver and muscle of juvenile pearl gentian groupers (Epinephelus fuscoguttatus × Epinephelus lanceolatus ). Six isonitrogenous and isolipidic experimental diets containing 0.04%, 0.17%, 0.35%, 0.66%, 1.29%, and 2.16% ARA (dry matter basis) were prepared by supplementing ARA purified oil into a basal diet (designated as S1, S2, S3, S4, S5, and S6, respectively). The experimental diets were fed to juvenile pearl gentian groupers with an initial body weight of (23.77±0.98) g for 8 weeks, with three replicates per diet and 30 fish per replicate. The results showed that: 1) Both specific growth rate (SGR) and feed efficiency (FE) exhibited a trend of initially increasing and subsequently decreasing with elevated dietary ARA levels, reaching maximum values in the S4 group, which were significantly higher than those in the S1 group ($P<0.05$). Whole-body and hepatic crude lipid contents were lowest in the S3 group, significantly lower than those in the S5 and S6 groups ($P<0.05$). 2) Hepatic superoxide dismutase (SOD) and catalase (CAT) activities, as well as total antioxidant capacity (T-AOC), in the S4 group did not differ significantly from the S3 group ($P>0.05$) but were significantly higher than those in the S1 and S6 groups ($P<0.05$). Hepatic malondialdehyde (MDA) content in the S3 and S4 groups was significantly lower than that in the S1 and S6 groups ($P<0.05$). 3) Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP) activities were all lowest in the S4 group, significantly lower than those in the S1 and S6 groups ($P<0.05$). 4) Hepatic and

muscular C20:3n-6 and C20:4n-6 contents increased significantly with increasing dietary ARA levels (P<0.05), whereas C18:3n-6, C20:5n-3, and C22:6n-3 contents decreased to varying degrees. It is concluded that appropriate dietary ARA levels (0.35%~0.66%) can promote growth and enhance antioxidant capacity and liver health in juvenile pearl gentian groupers. Using SGR and FE as evaluation indices, broken-line model regression analysis determined that the optimal dietary ARA levels for juvenile pearl gentian groupers were 0.45% and 0.56% of diet dry weight, respectively.

## Full Text

# Effects of Dietary Arachidonic Acid Level on Growth Performance, Antioxidant Capacity, Serum Biochemical Indices, and Fatty Acid Composition in Liver and Muscle of Juvenile Hybrid Grouper (Epinephelus fuscoguttatus × Epinephelus lanceolatus )

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### Abstract

This study investigated the effects of dietary arachidonic acid (ARA) level on growth performance, antioxidant capacity, serum biochemical parameters, and fatty acid composition in liver and muscle of juvenile hybrid grouper (Epinephelus fuscoguttatus × Epinephelus lanceolatus ). Six isonitrogenous and isolipidic experimental diets were formulated with ARA levels of 0.04%, 0.17%, 0.35%, 0.66%, 1.29%, and 2.16% (dry matter basis) by adding purified ARA oil to a basal diet (designated S1, S2, S3, S4, S5, and S6, respectively). The diets were fed to juvenile hybrid grouper with an initial body weight of (23.77±0.98) g for 8 weeks, with three replicates per diet and 30 fish per replicate. The results showed that: (1) Specific growth rate (SGR) and feed efficiency (FE) increased initially and then decreased with rising dietary ARA levels, reaching maximum values in the S4 group, which were significantly higher than those in the S1 group (P<0.05). Whole-body and liver crude lipid contents were lowest in the S3 group, significantly lower than in the S5 and S6 groups (P<0.05). (2) Liver superoxide dismutase (SOD) and catalase (CAT) activities and total antioxidant capacity (T-AOC) in the S4 group showed no significant differences from the S3 group (P>0.05) but were significantly higher than in the S1 and S6 groups (P<0.05). Liver malondialdehyde (MDA)

content in the S3 and S4 groups was significantly lower than in the S1 and S6 groups ($P<0.05$). (3) Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AKP) activities were lowest in the S4 group, significantly lower than in the S1 and S6 groups ($P<0.05$). (4) Liver and muscle C20:3n-6 and C20:4n-6 contents increased significantly with increasing dietary ARA levels ($P<0.05$), while C18:3n-6, C20:5n-3, and C22:6n-3 contents decreased to varying degrees. These findings indicate that an appropriate dietary ARA level (0.35%~0.66%) promotes growth, enhances antioxidant capacity, and improves liver health in juvenile hybrid grouper. Based on SGR and FE using broken-line model regression analysis, the optimal dietary ARA levels were estimated to be 0.45% and 0.56% of diet dry weight, respectively.

**Keywords:** Hybrid grouper; Arachidonic acid; Growth performance; Serum biochemical indices; Fatty acid composition

## Introduction

Long-chain polyunsaturated fatty acids (LC-PUFA) play crucial roles in regulating fish growth performance, immune function, cell membrane structure, reproductive performance, and lipid metabolism [1-3]. Previous research on LC-PUFA in marine animals has primarily focused on n-3 LC-PUFA, particularly C22:6n-3 (DHA) and C20:5n-3 (EPA) [4-5]. Arachidonic acid (ARA), an important n-6 LC-PUFA, has been overlooked in fish nutrition because it is not dominant in fish tissues compared to DHA and EPA [6]. However, recent studies have revealed that ARA metabolism produces various highly bioactive eicosanoids, including prostaglandins (PGs), thromboxanes (TX), and leukotrienes (LTs) [7], which regulate important physiological processes and significantly impact growth, development, and immune function.

Research has demonstrated that dietary ARA promotes growth and survival in marine animals while affecting lipid deposition and fatty acid composition [8-9]. Additionally, ARA regulates antioxidant capacity and immune function, maintaining fish health [10-11]. Studies on turbot (Scophthalmus maximus) [12] and half-smooth tongue sole (Cynoglossus semilaevis) [13] have confirmed that ARA plays an important role in regulating non-specific immunity and enhances stress resistance in larvae and juveniles [14-15]. Research on sea cucumber (Apostichopus japonicus) also showed that ARA improves growth performance and antioxidant capacity while affecting body wall fatty acid composition [16]. Zuo et al. [17] reported that appropriate ARA levels promote growth and gonad development in sea urchin (Strongylocentrotus intermedius) and affect intestinal microbiota structure.

Hybrid grouper (Epinephelus fuscoguttatus × Epinephelus lanceolatus ), a hybrid of giant grouper male and tiger grouper female, exhibits rapid growth, delicious flesh, strong disease resistance, and high market value, making it a popular cultured species in Guangdong and Fujian coastal areas. Previous nu-

tritional studies on hybrid grouper have focused on protein and amino acid nutrition, fishmeal replacement, and mineral nutrition [18-21], with limited research on fatty acid nutrition and no reports on ARA nutrition. Therefore, this study investigated the effects of different dietary ARA levels on growth performance, antioxidant capacity, serum biochemical indices, and tissue fatty acid composition to provide baseline data for determining appropriate ARA supplementation levels in hybrid grouper feeds and to establish a theoretical foundation for understanding ARA' s effects on immunity and fatty acid metabolism.

### 1.1 Experimental Design and Diet Preparation

A basal diet was formulated using fish meal and casein as primary protein sources, wheat meal and -starch as main carbohydrate sources, supplemented with minerals, vitamins, and DHA-enriched oil (provided by Wuhan Cabio Bioengineering Co., Ltd.). Six isonitrogenous and isolipidic experimental diets with ARA levels of 0.04%, 0.17%, 0.35%, 0.66%, 1.29%, and 2.16% (dry matter basis) were prepared by adjusting the addition of purified ARA oil (provided by Wuhan Cabio Bioengineering Co., Ltd.) and balancing with tristearin . The diets were designated S1, S2, S3, S4, S5, and S6, with S1 as the control. The fatty acid composition of experimental diets is shown in . All feed ingredients were ground to pass through an 80-mesh sieve, mixed sequentially according to formulation ratios, then blended thoroughly with oil. Distilled water was added to achieve proper consistency, and the mixture was extruded into hard pellets of 2.5 mm and 3.5 mm diameter. The pellets were dried at approximately 50°C and stored in a ventilated, dry place.

### 1.2 Experimental Fish and Culture Management

The feeding trial was conducted at the Dongying Experimental Base of Shandong Marine Resource and Environment Research Institute. Juvenile hybrid grouper from the same batch produced at the base were acclimated to the experimental conditions for 15 days using the control diet (S1) before the trial. At the start of the experiment, fish were fasted for 24 h, and healthy juveniles with uniform size [average body weight (23.77±0.98) g] were randomly distributed into 18 culture tanks (75 cm diameter, 80 cm depth) at 30 fish per tank. Each diet was assigned to three replicate tanks in a recirculating aquaculture system. Fish were fed to satiation twice daily at 08:30 and 16:30. Uneaten feed was siphoned out 30 min after feeding and quantified. Water temperature was maintained at (27±1)°C, dissolved oxygen >6.2 mg/L, salinity 23.5-26.5, pH 7.5-8.0, and ammonia and nitrite concentrations <0.1 mg/L.

After the 8-week feeding trial, fish were fasted for 24 h, counted, and weighed. From each tank, eight fish were randomly sampled: three for whole-body proximate composition analysis (stored at -20°C) and five for tissue collection (liver, intestine, and muscle) which were immediately frozen in liquid nitrogen. Additionally, five fish per tank were sampled for blood collection via caudal vein. Blood was allowed to clot at 4°C for 4 h, then centrifuged at 3,000 r/min for

10 min, and serum was collected and frozen in liquid nitrogen. Another three fish per tank were measured for body length and weight to calculate condition factor (CF), then dissected to obtain liver and viscera for hepatosomatic index (HSI) and viscerosomatic index (VSI) calculations. All samples were stored at -80°C for subsequent analysis.

### 1.4.1 Growth Indices

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, %) = 100 × (final body weight - initial body weight) / dry feed intake
HSI (%) = 100 × liver wet weight / body weight
VSI (%) = 100 × viscera wet weight / body weight
CF (%) = 100 × body weight / body length$^3$ (body weight in g, body length in cm)

### 1.4.2 Serum Biochemical and Liver Antioxidant Indices

Serum biochemical parameters were measured using a Hitachi automatic biochemical analyzer (Model 7020, Japan), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), lactate dehydrogenase (LDH) activities, and albumin (ALB), triglyceride (TG), cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents. Liver antioxidant indices were determined using assay kits from Nanjing Jiancheng Bioengineering Institute, including total antioxidant capacity (T-AOC), catalase (CAT), superoxide dismutase (SOD) activities, and malondialdehyde (MDA) content.

### 1.4.3 Proximate Composition Analysis of Feed, Whole Body, and Tissues

Moisture content was determined by oven drying at 105°C to constant weight (GB/T 6435-2006). Crude protein was measured by the Kjeldahl method (GB/T 6432-2006). Crude lipid was extracted using the Soxhlet method (GB/T 6433-2006). Ash content was determined by incineration in a muffle furnace at 550°C (GB/T 6438-2007).

### 1.4.4 Fatty Acid Composition of Feed and Tissues

Fatty acid composition was analyzed by gas chromatography following Mourente et al. [22] with slight modifications. Approximately 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, 3

mL of 2 mol/L HCl-methanol solution was added and heated again at 75°C for 20 min. The mixture was then extracted with 1.5 mL of hexane (chromatographic grade) by vortexing and phase separation. 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 comparison with standard retention times, and relative contents were calculated by peak area normalization.

### 1.5 Statistical Analysis

Data are presented as mean ± standard error (mean±SE). One-way ANOVA was performed using SPSS 19.0 software, followed by Tukey' s multiple comparison test. Significance was set at $P<0.05$. Broken-line model analysis was used to estimate the ARA requirement based on SGR and FE.

## Results

### 2.1 Effects of Dietary ARA Level on Growth Performance of Juvenile Hybrid Grouper

As shown in , survival rates ranged from 91.11% to 100.00% with no significant differences among groups ($P>0.05$). SGR increased as dietary ARA level rose from 0.04% to 0.66%, peaking in the S4 group and significantly exceeding the S1 group ($P<0.05$). When ARA level increased further from 0.66% to 2.16%, SGR declined slightly but remained comparable to the S4 group ($P>0.05$). WGR and FE showed similar trends to SGR. HSI decreased initially then increased with rising ARA levels, with S3 and S4 groups significantly lower than other groups ($P<0.05$) and S6 significantly higher than all others ($P<0.05$). VSI reached its minimum in S3, significantly lower than other groups ($P<0.05$), while S5 and S6 showed significantly higher values ($P<0.05$). No significant differences were observed in CF among groups ($P>0.05$).

Broken-line model analysis indicated that maximum SGR was achieved at 0.45% dietary ARA [Figure 1: see original paper], while maximum FE occurred at 0.56% dietary ARA [Figure 2: see original paper].

### 2.2 Effects of Dietary ARA Level on Proximate Composition of Whole Body and Tissues

shows that whole-body crude lipid content decreased initially then increased with rising ARA levels, reaching its lowest value in S3, significantly lower than S5 and S6 ($P<0.05$) but not significantly different from S1, S2, and S4 ($P>0.05$). S6 had the highest whole-body crude lipid content, significantly exceeding all other groups ($P<0.05$). Whole-body crude protein showed the opposite trend, with S3 having the highest value, significantly greater than S1, S5, and S6 ($P<0.05$) but comparable to S2 and S4 ($P>0.05$). No significant differences were observed in whole-body moisture or ash content among groups ($P>0.05$).

Liver crude lipid content decreased from 28.21% to 20.43% as ARA increased from 0.04% to 0.35%, then rose to 29.01% as ARA further increased to 2.16%. S3 liver crude lipid was significantly lower than S1, S5, and S6 ($P<0.05$) but similar to S2 and S4 ($P>0.05$). Muscle crude lipid increased from 2.62% to 3.66% as dietary ARA rose from 0.04% to 2.16%, reaching its maximum in S6, significantly higher than S1, S2, and S3 ($P<0.05$).

### 2.3 Effects of Dietary ARA Level on Liver Antioxidant Indices of Juvenile Hybrid Grouper

reveals that liver SOD activity decreased initially then increased with rising ARA levels, with S3 and S4 groups significantly higher than other groups ($P<0.05$) but not significantly different from each other ($P>0.05$). Liver CAT activity and T-AOC peaked in S4, with T-AOC significantly higher than S1, S2, S5, and S6 ($P<0.05$), and CAT activity significantly higher than S1, S2, and S6 ($P<0.05$). Liver MDA content decreased significantly as ARA increased from 0.04% to 0.66% ($P<0.05$), then increased significantly as ARA rose further to 2.16% ($P<0.05$), reaching its maximum in S6, significantly higher than S3, S4, and S5 ($P<0.05$) but comparable to S1 and S2 ($P>0.05$).

### 2.4 Effects of Dietary ARA Level on Serum Biochemical Parameters of Juvenile Hybrid Grouper

shows that serum ALT activity decreased initially then increased with rising ARA levels, with S3 and S4 groups significantly lower than S1 and S6 ($P<0.05$). Serum AST, AKP, and LDH activities showed similar trends. Serum TG content decreased initially then increased, remaining relatively low at 0.35%-0.66% ARA but reaching its maximum at 2.16% ARA, significantly higher than all other groups ($P<0.05$). Serum CHOL content increased gradually with ARA level, peaking at 2.16% ARA, significantly higher than S1 ($P<0.05$) but not significantly different from other groups ($P>0.05$). Serum HDL-C content was relatively high in S3 and S4, significantly higher than S1 and S6 ($P<0.05$), while LDL-C showed the opposite trend.

### 2.5 Effects of Dietary ARA Level on Liver and Muscle Fatty Acid Composition of Juvenile Hybrid Grouper

and demonstrate that liver and muscle C20:3n-6, C20:4n-6, and n-6 PUFA contents increased significantly with rising dietary ARA ($P<0.05$), while C18:1n-9, C18:1n-7, C18:3n-6, C20:5n-3, and C22:6n-3 contents decreased to varying extents. No significant differences were observed in C14:0 and C16:0 contents among groups ($P>0.05$). Muscle C18:0 and C18:3n-3 decreased with increasing ARA, with S6 significantly lower than S1 and S2 ($P<0.05$), whereas liver C18:0 and C18:3n-3 showed no significant changes ($P>0.05$). Liver C18:2n-6 increased with ARA level, with S5 and S6 significantly lower than S1 and S2 ($P<0.05$), while muscle C18:2n-6 showed no significant variation ($P>0.05$).

## Discussion

### 3.1 Effects of Dietary ARA Level on Growth Performance of Juvenile Hybrid Grouper

This study demonstrated that appropriate dietary ARA levels (0.35%-0.66%) enhanced WGR, SGR, and FE in juvenile hybrid grouper, indicating that this species requires a certain amount of ARA for normal growth and physiological function. These findings align with previous studies on other fish species. Xu et al. [6] reported optimal growth in Japanese seabass (Lateolabrax japonicus) juveniles at 0.36%-0.56% dietary ARA. Furuita et al. [23] found that Japanese flounder (Paralichthys olivaceus) broodstock required 0.6% ARA. Wang et al. [9] determined the ARA requirement for larger Japanese seabass [(207.16±0.72) g] to be 0.37% based on SGR and FE. Studies on Japanese flounder larvae [24], European seabass (Dicentrarchus labrax) juveniles [25], and half-smooth tongue sole larvae [26] also indicated higher ARA requirements for marine larvae and juveniles. However, some studies reported no significant growth-promoting effects of ARA [8, 27], possibly due to differences in species, size, culture conditions, and methodologies.

The dose-dependent effect of ARA on hybrid grouper growth was evident, with optimal performance at 0.66% ARA and impaired growth at both deficient and excessive levels. Similar results have been reported for Japanese seabass [6,9] and turbot [28]. Excessive ARA may inhibit development by suppressing EPA bioconversion, and imbalanced DHA/EPA ratios can affect normal growth and mask ARA's nutritional benefits [23]. Analysis of morphological indices revealed that HSI and VSI peaked in S6 (the highest ARA group), significantly exceeding other groups. This suggests that excessive ARA may cause liver damage and metabolic impairment, or lead to incomplete ARA absorption and excessive fat deposition [12,29]. Similar findings were reported in Japanese seabass [6,9] and cobia (Rachycentron canadum) [30].

### 3.2 Effects of Dietary ARA Level on Proximate Composition of Whole Body and Tissues

Whole-body and liver crude lipid contents were lowest in S3, significantly lower than in S5 and S6, and liver crude lipid in S3 was significantly lower than in S1. This indicates that appropriate ARA levels can effectively reduce lipid content in fish body and liver, though the underlying mechanism requires further investigation. These results are consistent with Wang et al. [29] in Japanese seabass, where dietary ARA at 1.38%-2.32% significantly downregulated expression of lipid oxidation-related genes (PPAR- and CPT-I) and increased whole-body and liver crude lipid contents, while 0.37%-0.60% ARA resulted in lower lipid contents. Tian et al. [8] also reported lowest body crude lipid content in grass carp at 0.30% dietary ARA, significantly lower than at 0.03% and 0.60% ARA. These findings suggest that appropriate ARA reduces body and tissue lipid content, while ARA deficiency or excess disrupts normal lipid metabolism and

causes excessive fat deposition.

### 3.3 Effects of Dietary ARA Level on Antioxidant Capacity of Juvenile Hybrid Grouper

Studies have confirmed that appropriate dietary ARA enhances fish antioxidant capacity. As the primary metabolic organ, liver antioxidant capacity indirectly reflects whole-body antioxidant status [31]. Antioxidant capacity comprises enzyme activities (T-AOC, SOD, etc.) reflecting the integrated antioxidant system function, and metabolic products (MDA, etc.) indicating the severity of free radical attack [32-33]. In this study, liver T-AOC, SOD, and CAT activities peaked in S4, significantly higher than in S1 and S6, while liver MDA content was lowest in S4, significantly lower than in S1 and S6. This demonstrates that appropriate ARA levels (0.35%-0.66%) enhance liver antioxidant capacity in juvenile hybrid grouper, confirming ARA' s regulatory role in antioxidant function. The dose-dependent relationship between ARA and antioxidant capacity was evident, as both deficient and excessive ARA levels reduced antioxidant capacity and compromised liver health, consistent with findings in turbot [28], Japanese seabass [6,29], and sea cucumber [16].

### 3.4 Effects of Dietary ARA Level on Serum Biochemical Parameters of Juvenile Hybrid Grouper

Under normal conditions, serum AST and ALT activities are low but increase when liver cells are damaged or permeability changes, making these indicators useful for assessing liver health and overall condition [34-35]. The significantly lower AST and ALT activities in S3 and S4 groups suggest that appropriate ARA levels benefit liver health in hybrid grouper, consistent with studies on turbot [28] and grass carp [8]. AKP is an important metabolic regulatory enzyme that hydrolyzes phosphate esters in alkaline environments and plays a crucial role in non-specific immunity [36]. The similar trend of serum AKP activity to AST and ALT indicates that 0.35%-0.66% dietary ARA enhances non-specific immunity. ARA affects immune function primarily by influencing eicosanoid production. ARA-derived eicosanoids, mainly 2-series prostaglandins and 4-series leukotrienes, regulate immune cell function [37]. Prostaglandin F2 promotes myofibril formation and limits muscle degradation, while prostaglandin E2 has opposite effects [38]. Therefore, different ARA levels may alter eicosanoid content or the PGE2/PGF2 ratio, differentially modulating immune responses. Li et al. [39] demonstrated that ARA significantly affects immune and physiological activities of head kidney macrophages in large yellow croaker.

Previous studies reported that dietary n-3 PUFA reduces hepatic very low-density lipoprotein secretion and increases chylomicron metabolism and clearance, thereby decreasing serum TG [40-41], while excessive n-6 PUFA may trigger inflammation and liver damage, causing lipid metabolism disorders and elevated TG and LDL-C [42]. In this study, serum TG, CHOL, and LDL-C peaked in S6 (the highest ARA group), suggesting that high ARA levels may

burden liver metabolism and cause lipid metabolism disorders. HDL-C transports cholesterol back to the liver, and higher serum HDL-C indicates better cardiovascular health. Notably, S3 and S4 groups showed significantly higher HDL-C than S1 and S6, confirming better health status in these groups. Combined with whole-body and liver lipid data, these results suggest that appropriate ARA levels positively affect lipid metabolism, while excessive or deficient ARA disrupts normal lipid metabolism and causes fat accumulation, warranting further mechanistic investigation.

### 3.5 Effects of Dietary ARA Level on Tissue Fatty Acid Composition of Juvenile Hybrid Grouper

Numerous studies have shown that fish tissue fatty acid composition reflects dietary fatty acid composition, which was confirmed in this study. Liver and muscle n-6 PUFA (C18:2n-6, C18:3n-6, C20:3n-6, and C20:4n-6) contents mirrored dietary changes, while muscle C18:2n-6 showed less pronounced changes, possibly due to low deposition rates. Saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in liver and muscle showed different trends from dietary levels, likely because PUFA are preferentially retained while SFA and MUFA are preferentially utilized [43]. Liver and muscle EPA contents showed opposite trends to ARA, similar to findings in half-smooth tongue sole [26], Japanese seabass [6], and turbot [28]. This may reflect competitive interactions between EPA and ARA, where high ARA levels inhibit EPA incorporation into tissues [41].

## Conclusion

In conclusion, under the conditions of this study, appropriate dietary ARA levels (0.35%-0.66%) promoted growth, enhanced antioxidant capacity, and improved liver health in juvenile hybrid grouper. Based on broken-line model analysis using SGR and FE as criteria, the optimal dietary ARA levels were estimated to be 0.45% and 0.56% of diet dry weight, respectively.

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