

## Postprint: Optimal Dietary Vitamin C Content for Juvenile Spotted Halibut (*Verasper variegatus*)

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### Abstract

This study investigated the effects of dietary vitamin C levels on growth, digestion, absorption, antioxidant capacity, and immune function in juvenile spotted halibut (*Verasper variegatus*) to determine the optimal dietary vitamin C requirement. The experiment was conducted at a water temperature of  $(12.5 \pm 1.5)^\circ\text{C}$ , using healthy juvenile spotted halibut with a body weight of  $(38.0 \pm 0.8)$  g, which were randomly divided into 7 groups (3 replicates per group, 30 fish per replicate) and fed experimental diets containing vitamin C at 10.2, 249.1, 402.8, 616.2, 769.5, 909.4, and 1,177.8 mg/kg. The trial lasted for 8 weeks. The results showed that the 616.2 mg/kg vitamin C group exhibited the highest weight gain rate, feed efficiency, and feeding rate; its weight gain rate was significantly higher than that of the 10.2 and 909.4 mg/kg vitamin C groups ( $P < 0.05$ ), feed efficiency was significantly higher than all other groups ( $P < 0.05$ ), and feeding rate was significantly higher than all other groups except the 1,177.8 mg/kg vitamin C group ( $P < 0.05$ ). The dietary vitamin C level required to achieve maximum vitamin C accumulation in liver and muscle tissues was 769.5 mg/kg. The serum malondialdehyde content in the 616.2 and 769.5 mg/kg vitamin C groups was significantly lower than in all other groups except the 402.8 mg/kg vitamin C group ( $P < 0.05$ ); the serum catalase and lysozyme activities in the 402.8 mg/kg vitamin C group were the highest, significantly higher than all other groups ( $P < 0.05$ ); the serum superoxide dismutase activity in the 402.8, 616.2, and 769.4 mg/kg vitamin C groups was significantly higher than in all other groups ( $P < 0.05$ ). When dietary vitamin C levels were 402.8, 616.2, or 769.4 mg/kg, protease, amylase, and lipase activities in the stomach and intestine were all at relatively high levels. Dietary vitamin C levels between 249.1 and 616.2 mg/kg could increase intestinal villus height, enhance intestinal wall thickness, and promote intestinal mucosal growth. The results demonstrated that dietary vitamin C levels between 402.8

and 616.2 mg/kg could effectively meet the growth requirements of juvenile spotted halibut while improving antioxidant capacity, non-specific immunity, and digestive absorption ability.

## Full Text

### A Study on Optimal Dietary Vitamin C Content for Juvenile Spotted Halibut (*Verasper variegates*)

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## Abstract

This experiment investigated the effects of dietary vitamin C content on growth performance, digestive function, absorption capacity, antioxidant capability, and immune function of juvenile spotted halibut (*Verasper variegates*) to determine the optimal dietary vitamin C requirement. The trial was conducted at a water temperature of  $(12.5 \pm 1.5)^\circ\text{C}$ . *Healthy juveniles spotted halibut with an initial body weight of  $(38.0 \pm 0.8)$  g* were randomly allocated into seven groups (three replicates per group, 30 fish per replicate) and fed seven experimental diets containing vitamin C at 10.2, 249.1, 402.8, 616.2, 769.5, 909.4, and 1,177.8 mg/kg for eight weeks. The results showed that the 616.2 mg/kg vitamin C group achieved the highest weight gain rate, feed efficiency ratio, and feeding rate. The weight gain rate of this group was significantly higher than that of the 10.2 and 909.4 mg/kg vitamin C groups ( $P < 0.05$ ), feed efficiency ratio was significantly higher than all other groups ( $P < 0.05$ ), and feeding rate was significantly higher than all groups except the 1,177.8 mg/kg vitamin C group ( $P < 0.05$ ). Maximal vitamin C accumulation in liver and muscle tissues occurred at a dietary vitamin C content of 769.5 mg/kg. Serum malondialdehyde (MDA) content in the 616.2 and 769.5 mg/kg vitamin C groups was significantly lower than in all other groups except the 402.8 mg/kg vitamin C group ( $P < 0.05$ ). Serum catalase (CAT) and lysozyme activities peaked in the 402.8 mg/kg vitamin C group, significantly exceeding all other groups ( $P < 0.05$ ). Serum superoxide dismutase (SOD) activity in the 402.8, 616.2, and 769.4 mg/kg vitamin C groups was significantly higher than in other groups ( $P < 0.05$ ). Protease, amylase, and lipase activities in stomach and intestine were maintained at high levels when dietary vitamin C content was 402.8, 616.2, or 769.4 mg/kg. Dietary vitamin C levels between 249.1 and 616.2 mg/kg enhanced intestinal fold height, increased intestinal wall thickness, and promoted intestinal mucosal growth. These results indicate that dietary vitamin C content ranging from 402.8 to 616.2 mg/kg effectively meets the

growth requirements of juvenile spotted halibut while improving antioxidant capacity, non-specific immunity, and digestive absorption ability.

**Keywords:** juvenile spotted halibut (*Verasper variegates*); vitamin C; growth; immunity; antioxidant

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## Introduction

Vitamin C, also known as L-ascorbic acid, is an essential micronutrient for animal growth, production, and maintenance of normal physiological functions. It regulates lipid metabolism, maintains normal cardiac, central nervous, and hematopoietic functions, and plays a crucial role in synthesizing various hormones. Vitamin C also promotes fish growth, alleviates environmental stress responses, and enhances immunity [1]. Most fish cannot synthesize vitamin C due to the lack of L-gulonolactone oxidase, the enzyme required for vitamin C synthesis, and must therefore obtain it from their diet [2]. Vitamin C deficiency in fish commonly leads to spinal and cartilage deformities, collagen damage, and hemorrhaging in skin and internal organs.

Numerous studies have reported on vitamin C's effects on fish physiological functions. Supplementing feed with high levels of vitamin C can enhance fish immunity and accelerate wound healing. Wan et al. [3] demonstrated that high dietary vitamin C significantly improved the immunity of juvenile blunt snout bream. Zhou et al. [4] reported that when vitamin C supplementation reached 396.4 mg/kg, serum total complement content and lysozyme activity were elevated in juvenile Japanese seabass. Song et al. [1] and Eo et al. [5] reported that vitamin C significantly enhanced the tolerance to environmental pollutants and pathogens as well as non-specific immunity in gibel carp and tiger puffer, respectively. Researchers have widely utilized vitamin C to improve disease resistance in aquatic animals. Spotted halibut (*Verasper variegates*) is an important economically cultured fish species, and enhancing its antioxidant and immune functions is significant for aquaculture. However, no studies have yet investigated the effects of vitamin C on antioxidant and immune functions in juvenile spotted halibut. This study examined growth performance, digestive enzyme activity, serum biochemical indices, intestinal morphology, and tissue vitamin C accumulation in juvenile spotted halibut after eight weeks of feeding diets with varying vitamin C levels to determine the optimal vitamin C requirement for promoting growth and enhancing immunity and antioxidant capacity, thereby providing data for fish immunology and nutritional research and a theoretical basis for vitamin C application in aquaculture.

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### 1.1 Experimental Diets

This experiment employed a single-factor design using fish meal and casein as protein sources and fish oil and soybean oil as lipid sources to formulate a basal diet, with composition and nutrient levels shown in Table 1. Fish meal, soybean meal, wheat meal, krill meal, beer yeast, and fish oil were purchased from Qingdao Qihao Biological Technology Co., Ltd. Carboxymethyl cellulose and microcrystalline cellulose were obtained from Linxia Huaan Biological Products Co., Ltd. Phospholipids, premix, choline, and calcium dihydrogen phosphate were sourced from Qingdao Jinhaiali Aquatic Technology Co., Ltd. Vitamin C polyphosphate (effective vitamin C content 35%) from Qingdao Jinhaiali Aquatic Technology Co., Ltd. served as the vitamin C source. Following Xie et al. [6], 0, 1,500, 3,000, 4,500, 6,000, 7,500, and 9,000 mg/kg of vitamin C polyphosphate were added to the basal diet to prepare seven experimental diets with measured vitamin C contents of 10.2, 249.1, 402.8, 616.2, 769.5, 909.4, and 1,177.8 mg/kg, respectively. Feed ingredients were ground to pass through a 60-mesh sieve, mixed thoroughly, and processed into 5 mm pellets, which were dried and stored at -20 °C until use.

### 1.2 Experimental Animals and Husbandry

Juvenile spotted halibut were obtained from Yantai Tianyuan Aquatic Co., Ltd. Healthy juveniles from the same batch with body weight of  $(38.0 \pm 0.8)$  g were randomly divided into seven groups with three replicates each (30 fish per replicate), with no significant difference ( $P > 0.05$ ). Fish were acclimated in cylindrical tanks (53 cm diameter, 60 cm height) for one week and fasted for 24 hours before the experiment, with feed intake recorded. Continuous aeration maintained adequate dissolved oxygen levels. The environmental temperature was  $(12.5 \pm 1.5)$  °C, pH was  $7.8 \pm 0.2$ , and dissolved oxygen content was above 6.0 mg/L. The experimental period lasted eight weeks.

### 1.3 Sample Collection and Measurements

At the beginning and end of the trial, fish in each tank were counted and weighed to calculate growth indices. Five fish were randomly selected from each tank and approximately 2 mL of blood was collected from the caudal vein using 1% heparin sodium as anticoagulant. After standing at low temperature for 4 hours, blood was centrifuged at 4,000 r/min for 10 minutes, and serum was separated and stored at -20 °C for determination of catalase (CAT), superoxide dismutase (SOD), lysozyme activities, and malondialdehyde (MDA) content. Fish were then dissected to isolate stomach and intestine for protease, amylase, and lipase activity measurements. Muscle samples were collected for nutrient composition analysis. Intestine from one fish per tank was fixed in Bouin's solution for routine paraffin sectioning at 6 μm thickness and stained with hematoxylin-eosin (HE). Five or more complete fold heights, mucosal thicknesses, and intestinal wall thicknesses were measured per intestinal sample using an ocular micrometer and averaged. Microscopic observation and photography were performed (10×). Feed and muscle samples were dried to constant weight at 105 °C to determine

dry matter content. Crude ash content was determined by weight loss after ignition at  $(550 \pm 20)$  °C for 5 hours in a muffle furnace. Crude protein content was measured by the Kjeldahl method (VELP UDK142 Automatic Distillation Unit, Italy). Crude lipid content was determined by Soxhlet extraction using petroleum ether as solvent (SOXTEC2050 FOSS Fat Analyzer, Sweden). Enzyme activities were measured using kits from Nanjing Jiancheng Bioengineering Institute. Vitamin C content was determined by the 2,4-dinitrophenylhydrazine method.

Growth indices were calculated as follows:

Weight gain rate =  $100 \times (\text{final mean body weight} - \text{initial mean body weight}) / \text{initial mean body weight}$

Survival rate =  $100 \times \text{final fish number} / \text{initial fish number}$

Feed efficiency ratio (FER) =  $100 \times \text{total weight gain} / \text{total feed intake}$

Specific growth rate (SGR) =  $100 \times (\ln \text{final mean body weight} - \ln \text{initial mean body weight}) / \text{feeding days}$

Feeding rate (FR) =  $100 \times \text{total feed intake} / [(\text{initial total body weight} + \text{final total body weight}) / 2 \times \text{feeding days}]$

## 1.5 Statistical Analysis

Experimental results are expressed as mean  $\pm$  standard deviation. One-way ANOVA was performed using SPSS 17.0 software.

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## Results

### 2.1 Growth Performance

The effects of dietary vitamin C content on growth performance of juvenile spotted halibut are presented in Table 2. After 56 days of culture, juvenile spotted halibut showed favorable growth, with body weight increasing from 38.8 g to 84.6 g (110% increase). No significant differences were observed in specific growth rate or survival rate among groups ( $P > 0.05$ ). With increasing dietary vitamin C content, feeding rate, weight gain rate, and feed efficiency ratio initially increased; however, when vitamin C content reached or exceeded 616.2 mg/kg, a growth plateau or inhibitory decline occurred. Weight gain rates in the 249.1, 402.8, 616.2, 769.5, and 1,177.8 mg/kg vitamin C groups were significantly higher than in the 10.2 and 909.4 mg/kg groups ( $P < 0.05$ ). Feed efficiency ratio in the 616.2 mg/kg vitamin C group was significantly higher than all other groups ( $P < 0.05$ ). Feeding rate in the 616.2 mg/kg vitamin C group was significantly higher than all groups except the 1,177.8 mg/kg group ( $P < 0.05$ ). Based on Table 2 data, the dietary vitamin C content for maximal growth of juvenile spotted halibut was 616.2 mg/kg.

## 2.2 Muscle Nutrient Composition

The effects of dietary vitamin C content on muscle nutrient composition are shown in Table 3. Dietary vitamin C content did not significantly affect muscle moisture content ( $P>0.05$ ). Crude ash content in the 249.1 and 402.8 mg/kg vitamin C groups was significantly higher than in all other groups except the 909.4 mg/kg group ( $P<0.05$ ). No significant differences in crude protein content were observed among the 249.1, 402.8, 616.2, and 769.5 mg/kg vitamin C groups ( $P>0.05$ ), but all were significantly higher than the 10.2, 909.4, and 1,177.8 mg/kg groups ( $P<0.05$ ). Muscle crude lipid content increased significantly as dietary vitamin C increased from 10.2 to 402.8 mg/kg ( $P<0.05$ ), then decreased significantly when vitamin C content reached 769.5 mg/kg ( $P<0.05$ ).

## 2.3 Tissue Vitamin C Accumulation

As shown in Figure 1 [Figure 1: see original paper], vitamin C content in liver, muscle, and serum increased significantly with dietary vitamin C content ( $P<0.05$ ) before plateauing. Figures 1-A and 1-B show that vitamin C accumulation in liver and muscle reached saturation at 769.5 mg/kg dietary vitamin C; at 1,177.8 mg/kg, liver vitamin C content decreased but remained significantly higher than levels at 10.2, 249.1, 402.8, and 616.2 mg/kg ( $P<0.05$ ). Serum vitamin C content plateaued when dietary vitamin C reached 909.4 mg/kg (Figure 1-C). Collectively, these results indicate that maximal tissue vitamin C accumulation in juvenile spotted halibut occurred at 769.5 mg/kg dietary vitamin C.

## 2.4 Serum Antioxidant System and Lysozyme Activity

Figure 2 [Figure 2: see original paper]-A shows that serum MDA content decreased initially with increasing dietary vitamin C, reaching a nadir at 616.2 and 769.5 mg/kg, which was significantly lower than all groups except the 402.8 mg/kg group ( $P<0.05$ ). When dietary vitamin C exceeded 769.5 mg/kg, MDA content began to increase. Serum CAT activity peaked at 402.8 mg/kg dietary vitamin C (9.05 U/mL), significantly higher than all other groups ( $P<0.05$ ); beyond 402.8 mg/kg, CAT activity was inhibited and declined, though remaining significantly higher than the 10.2 mg/kg group ( $P<0.05$ ). Serum SOD activity was elevated and plateaued at 402.8, 616.2, and 769.4 mg/kg dietary vitamin C; activity decreased significantly when dietary vitamin C exceeded 769.4 mg/kg ( $P<0.05$ ) but remained significantly higher than the 10.2 mg/kg group ( $P<0.05$ ). Serum lysozyme activity increased initially then decreased with rising dietary vitamin C, peaking at 402.8 mg/kg and significantly exceeding all other groups ( $P<0.05$ ); activity was inhibited and declined when dietary vitamin C exceeded 402.8 mg/kg.

## 2.5 Digestive Enzyme Activities

The effects of dietary vitamin C on amylase and protease activities are presented in Table 4 . Stomach and intestinal protease activities increased initially then decreased with rising dietary vitamin C. Stomach protease activity was highest in the 769.5 mg/kg vitamin C group, significantly higher than the 10.2, 249.1, and 402.8 mg/kg groups ( $P < 0.05$ ). Intestinal protease activity peaked in the 616.2 mg/kg vitamin C group, significantly higher than all groups except the 769.5 mg/kg group ( $P < 0.05$ ). Stomach amylase activity reached maximum at 402.8 mg/kg dietary vitamin C and did not change significantly with further vitamin C increase ( $P > 0.05$ ), though it declined at 1,177.8 mg/kg while remaining significantly higher than the 10.2 mg/kg group ( $P < 0.05$ ). Intestinal amylase activity increased initially then decreased with rising dietary vitamin C, peaking at 616.2 mg/kg.

The effects of dietary vitamin C on lipase activity are shown in Table 5 . Stomach and intestinal lipase activities increased initially then decreased with rising dietary vitamin C. Stomach lipase activity was highest at 616.2 mg/kg dietary vitamin C, with no significant differences from the 249.1, 402.8, and 769.5 mg/kg groups ( $P > 0.05$ ); activity declined when vitamin C exceeded 769.5 mg/kg, showing no significant difference from the 10.2 mg/kg group ( $P > 0.05$ ). Intestinal lipase activity peaked in the 402.8 mg/kg vitamin C group, significantly higher than all groups except the 616.2 mg/kg group ( $P < 0.05$ ).

## 2.6 Intestinal Morphology

The effects of dietary vitamin C on intestinal microscopic structure parameters are presented in Table 6 . Fold height and intestinal wall thickness in the 402.8, 616.2, and 769.5 mg/kg vitamin C groups were significantly higher than in other groups ( $P < 0.05$ ). Although fold height in the 909.4 and 1,177.8 mg/kg groups was significantly lower than the aforementioned groups ( $P < 0.05$ ), it remained significantly higher than the 10.2 mg/kg group ( $P < 0.05$ ). No significant differences in intestinal wall thickness were observed among the 10.2, 249.1, 909.4, and 1,177.8 mg/kg groups ( $P > 0.05$ ). Mucosal thickness increased initially then decreased with rising dietary vitamin C, with the 616.2 and 769.5 mg/kg groups significantly higher than all others ( $P < 0.05$ ). No significant differences were found among the 402.8, 909.4, and 1,177.8 mg/kg groups ( $P > 0.05$ ), though all were significantly higher than the 10.2 mg/kg group ( $P < 0.05$ ).

The effects of dietary vitamin C on intestinal morphology (mid-intestine) are shown in Figure 3 [Figure 3: see original paper]. The 10.2 mg/kg vitamin C group exhibited thin intestinal walls, coarse and sparsely arranged folds, and thin villous mucosa (Figure 3- ). As dietary vitamin C increased gradually, intestinal wall thickness increased, folds became slender and densely arranged, villous thickness increased, and epithelial cells remained intact (Figures 3- , , , ). When dietary vitamin C exceeded 769.5 mg/kg, folds became coarse, short, and sparse, and intestinal wall thickness decreased (Figures 3- , , ).

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## Discussion

### 3.1 Effects on Growth Performance

Previous studies have reported that appropriate dietary vitamin C levels can effectively promote fish growth, whereas excessive levels may inhibit growth [7]. Due to low water temperature during winter, the maximum weight gain rate in this trial was 110%, which is relatively low compared to industrial aquaculture operations. Dietary vitamin C content did not significantly affect survival rate, consistent with Zhang et al.'s [8] findings that vitamin C supplementation did not significantly affect survival in Japanese flounder. As dietary vitamin C increased, specific growth rate, weight gain rate, and feed efficiency ratio initially increased; however, when vitamin C content reached or exceeded 616.2 mg/kg, a growth plateau or inhibitory decline occurred. These results align with findings in Japanese seabass [9] and largemouth bass [10], demonstrating that appropriate vitamin C supplementation can improve growth performance in marine fish. Data indicate that maximal specific growth rate, weight gain rate, and feed efficiency ratio in juvenile spotted halibut were achieved at 616.2 mg/kg dietary vitamin C, which is lower than the optimal vitamin C requirement for growth in barfin flounder (0.1%-0.2%) [6] but higher than that for seabass [6] and topmouth culter [11], with effective requirements of 50.1 and 70.0 mg/kg, respectively.

### 3.2 Effects on Muscle Nutrient Composition

Results indicate that dietary vitamin C content did not significantly affect muscle moisture content in juvenile spotted halibut. Crude ash content was elevated in the 249.1 and 402.8 mg/kg vitamin C groups compared to other groups. Muscle crude protein content increased significantly with dietary vitamin C and declined when vitamin C reached 909.4 mg/kg, differing from findings in tilapia [9] and cobia [12] where crude protein and ash contents were unaffected by dietary vitamin C. Vitamin C serves as a cofactor for carnitine synthesis [13], which is closely associated with lipid metabolism. Vitamin C deficiency also impairs collagen synthesis, suggesting that vitamin C can regulate body composition to some extent. Discrepancies among studies may relate to experimental species, conditions, and vitamin C supplementation levels. Muscle crude lipid content in juvenile spotted halibut increased initially then decreased with rising dietary vitamin C, consistent with Zhou et al.'s [7] findings in grouper that vitamin C deficiency or excess reduced body lipid content, possibly because vitamin C participates in lipid metabolism, though the mechanism requires further investigation.

### 3.3 Effects on Tissue Vitamin C Accumulation

Vitamin C content in liver, serum, and muscle is commonly used to evaluate vitamin C nutritional status in fish [9]. Dabrowski et al. [14] suggested that the dietary vitamin C level producing stable tissue vitamin C content represents the optimal requirement. This study showed that tissue vitamin C content increased initially with dietary vitamin C and reached saturation in liver and muscle at 769.5 mg/kg. Liver vitamin C content was markedly higher than in muscle and serum, indicating that the liver is the primary site for vitamin C accumulation and metabolism. Serum vitamin C content increased continuously with dietary vitamin C, with large differences among groups, suggesting that serum vitamin C depends on dietary intake, consistent with Wen' s [15] report. Results indicate that maximal tissue vitamin C accumulation in juvenile spotted halibut occurred at 769.5 mg/kg dietary vitamin C.

### 3.4 Effects on Serum Antioxidant System and Lysozyme Activity

Animal antioxidant defense systems against excess free radicals include enzymatic and non-enzymatic systems. The enzymatic system primarily comprises antioxidant enzymes such as SOD and CAT, which effectively scavenge free radicals to maintain a balance between formation and elimination, preventing peroxidative damage. When antioxidant enzyme activity is insufficient, antioxidant capacity declines, impairing timely and effective removal of oxygen free radicals, which accelerates lipid peroxidation and increases MDA content [16]. This study showed that serum SOD and CAT activities peaked while MDA content reached its lowest at 402.8 mg/kg dietary vitamin C, indicating enhanced antioxidant capacity. This suggests that exogenous vitamin C can neutralize or eliminate MDA, reducing lipid oxidation. When dietary vitamin C exceeded 769.5 mg/kg, serum SOD and CAT activities declined, reducing free radical scavenging capacity and increasing lipid peroxidation products and MDA content. These results demonstrate that either excessive or insufficient dietary vitamin C is detrimental to antioxidant capacity in juvenile spotted halibut, consistent with findings by Zhou et al. [17] and Ming et al. [18].

Lysozyme is an important hydrolytic enzyme in the fish non-specific immune system. Phagocytes can engulf harmful substances because lysozyme hydrolyzes the acetylamino polysaccharide in peptidoglycan of Gram-positive bacterial cell walls, causing lysis and release of contents to eliminate invading bacteria and protect the organism [19-20]. This study showed that lysozyme activity peaked at 402.8 mg/kg dietary vitamin C and declined when vitamin C exceeded this level, similar to results reported for Pacific white shrimp [21], Japanese seabass [4], and turbot [22]. This indicates that juvenile spotted halibut achieve maximal non-specific immunity at 402.8 mg/kg dietary vitamin C.

### 3.5 Effects on Digestive Enzyme Activities

Fish depend on gastrointestinal digestion and absorption of nutrients, with digestive enzyme activity serving as a key indicator of gastrointestinal digestive capacity. Fish regulate enzyme synthesis and secretion in response to changing nutritional conditions to adapt metabolism accordingly. This study showed that protease, amylase, and lipase activities increased initially then decreased with rising dietary vitamin C, positively correlating with rapid growth. Dietary vitamin C supplementation improved gastrointestinal digestive enzyme activity in spotted halibut, thereby promoting nutrient digestion and absorption, consistent with Ye et al. [23]. When dietary vitamin C exceeded 769.5 mg/kg, stomach and intestinal protease and amylase activities declined, coinciding with the decline node for growth performance and serum antioxidant enzyme activity, indicating that excessive vitamin C inhibits physiological indices in juvenile spotted halibut. Similar patterns have been reported for pantothenic acid [24] and niacin [25] in juvenile Jian carp. Stomach and intestinal lipase activities peaked at 402.8 mg/kg dietary vitamin C and were negatively correlated with vitamin C content beyond this level, opposite to findings in omnivorous common carp [26], possibly because high dietary protein levels provided sufficient protein for energy supply and deposition, reducing lipid requirement. This study suggests that the minimum dietary vitamin C content to promote digestion and absorption in juvenile spotted halibut is 616.2 mg/kg.

### 3.6 Effects on Intestinal Morphology

Animals absorb nutrients primarily through the intestine. Aquatic animal intestines generally lack villi but possess folds, with absorptive epithelial cells distributed on the folds. Studies indicate that fish intestinal fold height reflects nutrient absorption capacity [27]; greater fold height and number increase absorptive surface area. Intestinal wall thickness affects nutrient absorption efficiency [28], while mucosal thickness reflects intestinal developmental maturity [29]. Histological observation of juvenile spotted halibut intestine revealed that fold height, mucosal thickness, and intestinal wall thickness in the 249.1, 402.8, and 616.2 mg/kg vitamin C groups were significantly higher than other groups, indicating that appropriate dietary vitamin C effectively promoted intestinal fold height and mucosal thickness. Other groups showed shorter, sparsely arranged folds and thinner mucosa and intestinal walls, suggesting that vitamin C deficiency or excess is detrimental to intestinal development and nutrient absorption. This study indicates that dietary vitamin C content between 249.1 and 616.2 mg/kg promotes intestinal development and nutrient absorption in juvenile spotted halibut. While few studies have examined vitamin C effects on intestinal morphology in marine fish, findings in juvenile Jian carp [30-31] are consistent with these results.

Based on these results, dietary vitamin C content of 616.2 mg/kg promoted growth, 769.5 mg/kg achieved maximal tissue vitamin C accumulation, 402.8 mg/kg provided maximal antioxidant capacity and non-specific immunity, 616.2

mg/kg enhanced nutrient digestion, and 249.1-616.2 mg/kg promoted intestinal development and nutrient absorption. Considering aquaculture economic benefits and disease prevention, the recommended practical dietary vitamin C content for juvenile spotted halibut is 402.8-616.2 mg/kg.

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

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