# Effects of Replacing Fish Meal with Antarctic Krill Meal on Growth Performance, Serum and Liver Biochemical Indices, and Serum Nonspecific Immune Indices in Juvenile Spotted Halibut (Verasper variegatus) Postprint

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## Abstract

To investigate the effects of Antarctic krill meal replacement of fish meal on growth performance, serum and liver biochemical parameters, and serum nonspecific immune indices in juvenile spotted halibut. A basal diet was formulated using fish meal and corn gluten meal as protein sources, high-gluten flour as carbohydrate source, and fish oil, soybean oil, and phospholipids as lipid sources. Antarctic krill meal was used to replace 0% (control), 10%, 20%, 30%, 40%, and 50% of fish meal in the basal diet to formulate six isonitrogenous and isolipidic experimental diets (designated as R0, R10, R20, R30, R40, and R50), which were fed to juvenile spotted halibut with an initial body weight of (38.16$\pm$0.11) g for 50 days. Each diet was assigned three replicates, with 30 fish per replicate. The results showed: 1) The weight gain rate and feed efficiency of juvenile spotted halibut in the R30 and R40 groups were significantly higher than those in other groups ($P<0.05$); the mortality rate in the R40 group was significantly higher than that in other groups ($P<0.05$). The level of fish meal replacement with Antarctic krill meal had no significant effect on hepatosomatic index and viscerosomatic index of juvenile spotted halibut ($P>0.05$). 2) Serum total protein content in the R10 and R30 groups was significantly higher than that in other groups ($P<0.05$); replacement of 10%-30% fish meal with Antarctic krill meal had no significant effect on serum aspartate aminotransferase and alanine aminotransferase activities, as well as liver glutamate dehydrogenase activity in juvenile spotted halibut ($P>0.05$). Serum aspartate aminotransferase and alanine aminotransferase activities in the R40 and R50 groups were significantly increased compared with the control group

(P<0.05), liver aspartate aminotransferase activity in the R40 group was significantly decreased compared with the control group (P<0.05), and liver alanine aminotransferase activity in the R50 group was significantly lower than that in other groups (P<0.05). 3) Replacement of 10%-30% fish meal with Antarctic krill meal significantly increased serum lysozyme and alkaline phosphatase activities in juvenile spotted halibut (P<0.05). In summary, replacement of 10%-30% fish meal with Antarctic krill meal can improve growth performance and non-specific immunity of juvenile spotted halibut, without adverse effects on liver function and protein metabolism.

## Full Text

## Effects of Antarctic Krill Meal Replacing Fish Meal on Growth Performance, Serum and Liver Biochemical Indices, and Serum Non-Specific Immune Indices of Juvenile Spotted Halibut (Verasper variegatus)

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### Abstract

This study investigated the effects of Antarctic krill meal (AKM) as a replacement for fish meal on growth performance, serum and liver biochemical indices, and serum non-specific immune indices in juvenile spotted halibut (*Verasper variegatus*). A basal diet was formulated using fish meal and corn gluten meal as protein sources, wheat gluten as a carbohydrate source, and fish oil, soybean oil, and phospholipid as lipid sources. Six isonitrogenous and isolipidic experimental diets were prepared by replacing 0% (control), 10%, 20%, 30%, 40%, and 50% of the fish meal in the basal diet with AKM, designated as R0, R10, R20, R30, R40, and R50, respectively. Each diet was fed to three replicate groups of 30 juvenile spotted halibut with an initial body weight of (38.16$±$0.11) g for 50 days. The results showed: (1) The R30 and R40 groups exhibited higher weight gain rate (WGR) and feed efficiency ratio (FER), significantly greater than other groups (P<0.05). The mortality rate in the R50 group was significantly higher than in other groups (P<0.05). Replacement level had no significant effect on hepatosomatic index (HSI) or viscerosomatic index (VSI) (P>0.05). (2) Serum total protein content in R10 and R30 groups was significantly higher than in other groups (P<0.05). Replacement of 10–30% fish meal with AKM had no significant effect on serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities or liver glutamate dehydrogenase (GDH) activity (P>0.05). However, serum GOT and GPT activities in R40 and R50 groups were significantly elevated compared to the control (P<0.05), while liver GOT activity in R40 group was significantly decreased (P<0.05), and liver GPT activity in R50 group was significantly lower than all other groups (P<0.05). (3) Replacement of 10–30% fish meal with AKM significantly increased serum lysozyme (LZM) and alkaline phosphatase (AKP) activities (P<0.05). Overall, AKM replacement of 10–30% fish meal improved growth performance and non-specific immunity in juvenile spotted halibut without adversely affecting liver function or protein metabolism.

**Keywords:** spotted halibut; Antarctic krill meal; growth performance; biochemical indices; non-specific immunity

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## Introduction

Spotted halibut (*Verasper variegatus*) is a valuable marine fish species in northern China, prized for its delicious flesh and high nutritional value, rich in essential amino acids and unsaturated fatty acids, and commanding significant economic value. Krill resources are abundant, representing one of the world' s largest marine biological reserves with tremendous development potential, and are considered the largest and last remaining protein reservoir on Earth. Antarctic krill meal exhibits excellent amino acid balance, high unsaturated fatty acid content, and is rich in carotenoids and astaxanthin, which positively influence animal growth, immunity, and body coloration. Numerous studies have investigated dietary krill meal supplementation, primarily focusing on Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), large yellow croaker (*Larimichthys croceus*), pearl gentian grouper ( *Epinephelus fuscoguttatus*× *Epinephelus lanceolatu*), and malabar grouper (*Epimephelus malabaricus*). Applications in flatfish species have only been reported for turbot (*Scophthalmus maximus*), half-smooth tongue sole (*Cynoglossus semilaevis*), Atlantic halibut (*Hippoglossus hippoglossus*), and starry flounder (*Platichthys stellatus*), with no reports on spotted halibut. Our research group previously conducted a qualitative evaluation of Antarctic krill meal as a protein source in flatfish feeds, and this study quantitatively assesses the effects of krill meal replacement on growth performance, liver and serum biochemical indices, and serum non-specific immune indices in juvenile spotted halibut to provide theoretical reference for krill meal application in flatfish aquafeeds.

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### 1.1 Experimental Diets

A basal diet was formulated using fish meal and corn gluten meal as protein sources, high-gluten flour as a carbohydrate source, and fish oil, soybean oil, and phospholipid as lipid sources. Six isonitrogenous and isolipidic experimental diets were prepared by replacing 0% (control), 10%, 20%, 30%, 40%, and 50% of the fish meal in the basal diet with Antarctic krill meal, designated as R0, R10, R20, R30, R40, and R50, respectively. Since krill meal contains higher lipid content than fish meal, fish oil and soybean oil were adjusted to maintain crude lipid content at approximately 8% across all six diets, resulting in gradually decreasing proportions of fish oil and soybean oil as replacement levels increased. Dietary composition and nutrient levels are presented in .

All ingredients were analyzed for nutrient composition, ground to pass through a 100-mesh sieve, and mixed according to formulation ratios. Phospholipid was dissolved in fish oil and soybean oil before mixing with other ingredients. Water (35% by weight) was added, and the mixture was extruded into 3 mm pellets, air-dried for 12–14 hours, and stored at -20 °C.

### 1.2 Feeding Management

Juvenile spotted halibut were obtained from Yantian Yuan Aquatic Co., Ltd. in Yantai, Shandong Province. Healthy, uniform-sized fish from the same batch with initial body weight of (38.16$\pm$0.11) $g were randomly distributed into 18 plastic tanks (53 cm \times 72 cm \times$ 60 cm), with 30 fish per tank. Each diet was fed to three replicate tanks. Prior to the experiment, fish were acclimated to the experimental conditions and fed the control diet for one week. During the 50-day trial, water temperature was maintained at approximately 14 °C, salinity at approximately 35, and dissolved oxygen concentration at approximately 5.5 mg/L. Water was exchanged daily at 08:30, and fish were fed to satiation once daily at 14:00. Uneaten feed was siphoned after 0.5 hours, dried, and weighed to record daily feed intake.

### 1.3 Sample Collection and Analysis

At the end of the experiment, fish were fasted for 24 hours before sampling. Fish in each tank were counted and weighed. Five fish were randomly selected from each tank, and approximately 3 mL of blood was collected from the caudal vein using heparin sodium (1% volume fraction) as anticoagulant. After standing at low temperature for 4 hours, blood was centrifuged at 4,000 r/min for 10 minutes. Viscera and liver were dissected and weighed to calculate viscerosomatic index (VSI) and hepatosomatic index (HSI). Serum was separated for analysis of total protein (TP) content and activities of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), and lysozyme (LZM).

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### 2.2 Effects of Antarctic Krill Meal Replacement on Serum and Liver Biochemical Indices

The effects of Antarctic krill meal replacement on serum and liver biochemical indices in juvenile spotted halibut are shown in . Serum total protein content in R10 and R30 groups was significantly higher than in the control group (P<0.05), while other groups were significantly lower (P<0.05). Compared with the control, replacement levels $ $30% had no significant effect on serum GOT and

GPT activities (P>0.05), whereas 40% and 50% replacement significantly reduced serum GOT and GPT activities (P<0.05). Liver GPT activity showed a decreasing trend with increasing replacement level, with all groups except R50 showing significantly higher activity than the control (P<0.05). Liver GOT activity in R30 group did not differ significantly from the control (P>0.05), while all other groups were significantly lower (P<0.05). Liver GDH activity was highest in R30 group, significantly higher than all groups except the control (P<0.05).

### 2.3 Effects of Antarctic Krill Meal Replacement on Serum Non-Specific Immune Indices

The effects of Antarctic krill meal replacement on serum non-specific immune indices in juvenile spotted halibut are presented in . All replacement groups showed significantly higher serum LZM and ACP activities than the control group (P<0.05). Additionally, all replacement groups except R10 exhibited significantly higher serum AKP activity compared to the control (P<0.05).

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### 3.1 Effects of Antarctic Krill Meal Replacement on Growth Performance

In this study, replacement of 10–40% fish meal with Antarctic krill meal improved weight gain rate and feed efficiency in juvenile spotted halibut. Numerous studies have reported on krill meal application in various fish species, with some indicating that high-level or complete replacement may reduce growth performance and feed efficiency, while moderate replacement levels may have no adverse effects or even promote growth. For instance, replacing 40% fish meal with Antarctic krill meal in Atlantic salmon diets and 60% in Atlantic halibut diets significantly improved specific growth rate. Research has also shown that 4% krill meal promotes growth and feed intake in malabar grouper, and replacement of 20–60% fish meal with krill meal had no negative effects on turbot growth. In the present study, WGR and FER increased initially then decreased with rising replacement levels, with optimal growth performance observed at 30% replacement. However, mortality increased significantly at 50% replacement, consistent with findings that krill meal replacement exceeding 30% reduced growth performance in starry flounder. Yoshitomi et al. reported that complete replacement of fish meal with krill meal adversely affected yellowtail growth. Hansen et al. found that complete replacement significantly reduced Atlantic salmon growth, whereas de-shelled low-fluoride krill meal improved growth performance. In this experiment, krill meal contained high fluoride levels (1,642.80$±$31.78 mg/kg), and dietary fluoride content increased with replacement level. The reduced growth performance and elevated mortality at 50% replacement may be attributed to fluoride toxicity, as excessive fluoride is harmful to fish. Additionally, krill meal contains substantial chitin, which is poorly digestible and may reduce digestive efficiency. Hepatosomatic and viscerosomatic indices were unaffected by replacement level, consistent with previous research. Variations in optimal replacement levels across fish species may relate to species differences, feeding habits, and environmental conditions, requiring further investigation.

### 3.2 Effects of Antarctic Krill Meal Replacement on Serum and Liver Biochemical Indices

Serum total protein maintains osmotic pressure and serves as an important indicator of protein metabolism, reflecting protein and amino acid digestion and absorption. Increased serum total protein enhances metabolism, benefiting protein synthesis, nitrogen deposition, and immune capacity. The significantly higher serum total protein in R10 and R30 groups suggests that appropriate krill meal levels promote protein utilization in juvenile spotted halibut. GOT and GPT primarily reside in liver tissue, with low serum activity under normal conditions. Elevated serum activity indicates tissue damage and altered membrane permeability, reflecting liver injury. The absence of significant differences in serum GPT and GOT activities in R10, R20, and R30 groups indicates that 10–30% replacement caused no liver damage or metabolic burden.

GDH is a key enzyme in non-essential amino acid synthesis, closely related to protein synthesis and degradation. Dietary amino acids are metabolized through transamination and deamination, with fish primarily utilizing combined deamination. GPT and GOT are key enzymes in amino acid metabolism, with activity levels reflecting both metabolic intensity and liver function. In this study, liver GDH activity did not differ significantly among replacement groups, indicating no adverse effects on protein metabolism. The highest liver GOT and GDH activities in R30 group suggest the most rapid protein metabolism without liver damage. Conversely, significantly reduced liver GOT activity in R40 group and liver GPT activity in R50 group indicate that excessive replacement levels may impair protein metabolism and liver function.

### 3.3 Effects of Antarctic Krill Meal Replacement on Serum Non-Specific Immune Indices

Aquatic animals lack secondary antibody responses in their specific immune mechanisms, making non-specific immunity critically important for defense. Lysozyme damages bacterial cell walls, with activity levels reflecting antibacterial capacity. Acid phosphatase (ACP), a lysosomal marker enzyme, participates in phosphate metabolism regulation, signal transduction, and energy conversion, while alkaline phosphatase (AKP) directly participates in phosphate group transfer and metabolism. In this study, all replacement groups showed significantly higher serum LZM activity than the control, consistent with findings that serum LZM activity in half-smooth tongue sole increased significantly with krill meal level. Replacement groups also exhibited elevated serum ACP and AKP activities, indicating that krill meal enhances these enzyme activities and improves non-specific immunity, likely due to its rich carotenoid and astaxanthin content. Astaxanthin is known to enhance immunity and antioxidant capacity, with studies reporting that dietary astaxanthin significantly improves immunity and antioxidant status in large yellow croaker and increases serum defensin content and LZM activity in rainbow trout.

In summary, Antarctic krill meal replacement of fish meal can improve weight gain rate and feed efficiency in juvenile spotted halibut, with optimal growth performance at 30% replacement level, though replacement exceeding 40% reduces survival. Replacement of 10–30% fish meal with krill meal has no adverse effects on liver function or protein metabolism while enhancing non-specific immunity in juvenile spotted halibut.

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