Effects of Replacing Fish Oil with Blended Vegetable Oil in Finishing Diets on Gonadal Development, Lipid Metabolism, Antioxidant Capacity, and Immune Function of Adult Male Chinese Mitten Crab (Eriocheir sinensis): Postprint

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Abstract

This experiment aimed to investigate the effects of replacing fish oil with mixed vegetable oil on gonadal development, lipid metabolism, antioxidant capacity, and immune performance in adult male Chinese mitten crabs (Eriocheir sinensis). Mixed vegetable oil (soybean oil:rapeseed oil=1:1) was used to replace fish oil at different levels (0, 25%, 50%, 75%, and 100%) to formulate five isonitrogenous and isolipidic fattening feeds (crude protein and crude lipid contents were 39.5% and 14.0%, respectively), designated as feeds 1#, 2#, 3#, 4#, and 5#. A 60-day outdoor pond feeding trial was conducted on five groups of adult male crabs (four replicates per group, 25 crabs per replicate). The results showed: 1) Replacement of fish oil with mixed vegetable oil at different levels had no significant effects on the gonadosomatic index (GSI) and hepatosomatic index (HSI) of male crabs ($P>0.05$). 2) The hepatopancreas TG content was lowest in feed 4# group, while TG and total cholesterol (TC) contents in hepatopancreas showed no significant differences among the other groups ($P>0.05$); Except for free cholesterol (FC) and high-density lipoprotein cholesterol (HDL-C) contents, all other serum lipid metabolism indices in feed 2# group were significantly higher than those in other groups ($P<0.05$). 3) For antioxidant indices in hepatopancreas, except for superoxide dismutase (SOD) activity which showed no significant difference among groups ($P>0.05$), the total antioxidant capacity (T-AOC), peroxidase (POD) activity, and malondialdehyde (MDA) content in feed 1# group were significantly higher than those in other groups ($P<0.05$); For immune indices in hepatopancreas, alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were highest in feed 1# group, while -glutamyl transpeptidase (-GT) activity was highest in feed 4# group, followed by feed

1# group, and lowest in feed 2# group. 4) Serum ALP activity was highest in feed 3# group, which was significantly higher than that in feeds 1# and 4# groups (P<0.05), while serum ACP activity showed no significant difference among groups (P>0.05); Serum hemocyanin (Hc) content, T-AOC, and SOD activity in feed 2# group were significantly higher than those in other groups (P<0.05), but POD activity and MDA content in serum were lowest in this group. In conclusion, replacement of fish oil with mixed vegetable oil (soybean oil:rapeseed oil=1:1) at different levels in fattening feeds had no significant effect on gonadal development of adult male Chinese mitten crabs, and appropriate replacement levels had no negative effects on lipid metabolism, antioxidant capacity, or immune performance of adult male Chinese mitten crabs, with the appropriate replacement level being 25%~50%.

Full Text

Effects of Fish Oil Replacement by Blending Vegetable Oils in Fattening Diets on Gonadal Development, Lipid Metabolism, Antioxidant and Immune Capacities of Adult Male Chinese Mitten Crab (*Eriocheir sinensis*)

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Abstract: This study investigated the effects of fish oil replacement by blending vegetable oils on gonadal development, lipid metabolism, and antioxidant and immune capacities of adult male Chinese mitten crab (*Eriocheir sinensis*). Five isonitrogenous and isolipidic fattening diets (39.5% crude protein and 14.0% crude lipid) were formulated to replace 0, 25%, 50%, 75% and 100% of fish oil with a blend of vegetable oils (soybean oil:rapeseed oil = 1:1), designated as diets 1#, 2#, 3#, 4# and 5#, respectively. Five groups of adult male crabs (four replicates per group, 25 crabs per replicate) were fed the experimental diets for 60 days in outdoor ponds. The results showed: 1) Replacement of fish oil at different levels had no significant effects on gonadosomatic index (GSI) and hepatosomatic index (HSI) of male crabs (P>0.05). 2) The lowest triglyceride (TG) content in hepatopancreas was observed in diet 4# group, while no significant differences were found in hepatopancreas TG and total cholesterol (TC) among the other groups (P>0.05). Except for free cholesterol (FC) and high-density lipoprotein cholesterol (HDL-C) contents, all other serum lipid metabolism indices in diet 2# group were significantly higher than those in other groups (P<0.05). 3) For hepatopancreas antioxidant indices, total antioxidant capacity (T-AOC), peroxidase (POD) activity and malondialdehyde (MDA) content in diet 1# group were significantly higher than those in other groups (P<0.05), while no significant difference was observed in superoxide dismutase (SOD) activity among all groups (P>0.05). For hepatopancreas immune indices, diet 1# group showed the highest alkaline phosphatase (ALP) and acid phosphatase (ACP) activities, whereas diet 4# group exhibited the highest -glutamyl transpeptidase (-GT) activity, followed by diet 1# group, and diet 2# group showed the lowest. 4) Serum ALP activity was highest in diet 3# group, significantly higher than that in diets 1# and 4# groups (P<0.05), but no significant difference was found in serum ACP activity among groups (P>0.05). Serum hemocyanin (Hc) content, T-AOC and SOD activity in diet 2# group were significantly higher than those in other groups (P<0.05), while this group showed the lowest serum POD activity and MDA content. In conclusion, replacement of fish oil by blending vegetable oils (soybean:rapeseed oil = 1:1) in fattening diets had no significant effect on male gonadal development, and appropriate replacement levels caused no negative effects on lipid metabolism, antioxidant and immune capacities of adult male *E. sinensis*. The appropriate replacement level is 25%–50%.

Keywords: fish oil replacement level; Chinese mitten crab (*Eriocheir sinensis*); gonadal development; lipid metabolism; antioxidant capacity

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Fish oil is one of the most important ingredients in aquafeeds, with approximately 75% of global fish oil production used for aquafeed manufacturing. With the rapid development of aquaculture, global fish oil resources can no longer meet the demands of aquafeed production, making it imperative to find suitable alternatives. Due to their wide availability, low cost and large production volume, vegetable oils have become the preferred choice for fish oil replacement in aquafeeds. Chinese mitten crab (*Eriocheir sinensis*), commonly known as river crab, is one of the major farmed crab species in China with high nutritional value. Its unique aroma and nutritional properties are associated with high levels of highly unsaturated fatty acids (HUFA), necessitating high dietary fish oil content during the gonadal development period. However, with increasing scarcity and rising prices of fish oil, vegetable oil substitution has become common in aquafeeds. Current research on fish oil replacement in Chinese mitten crab diets is limited and primarily focuses on the growth stage, with no studies examining the gonadal development stage. During this stage, gonadal development patterns differ significantly between females and males, and their nutritional requirements may also vary. Previous studies have shown that HUFA composition and content in fattening diets may affect female ovarian development, yet no research has been reported on fish oil replacement and HUFA requirements in male fattening diets, making it practically important to determine appropriate fish oil replacement levels. Considering that different vegetable oils have distinct fatty acid profiles, blending them may achieve fatty acid balance and improve replacement efficacy. Soybean and rapeseed oils are the most commonly used vegetable oils in aquafeeds, with complementary fatty acid and vitamin compositions, offering advantages when used in combination. Therefore, this study systematically investigated the effects of replacing fish oil at different levels (0, 25%, 50%, 75% and 100%) with blended vegetable oils (soybean oil:rapeseed oil = 1:1) in practical diets on gonadal development, lipid metabolism and antioxidant capacity of adult male crabs, aiming to provide theoretical and practical references for formulating fattening diets, fattening culture practices, and development of fish oil alternatives for Chinese mitten crab.

1.1 Experimental Diets

The experimental diets used soybean meal, rapeseed meal and fish meal as primary protein sources, with fish oil replaced at different levels by blended vegetable oils (soybean oil:rapeseed oil = 1:1). Five experimental diets were formulated according to the formula in Table 1 , with fish oil replacement levels of 0, 25%, 50%, 75% and 100%. Prior to diet preparation, all ingredients were ground to pass through a 60-mesh sieve (approximately 250 m diameter). Ingredients were first mixed using a Muyang SLHS2.0A single-shaft mixer, then subjected to secondary mixing with an SLHSJ1.0A double-shaft mixer. After thorough mixing, sinking extruded pellets were produced using a Hengrun HR118*2 twin-screw extruder (4.5–5.0 mm diameter, ~10.0 mm length). Vegetable and fish oils were added proportionally via vacuum coating. All experimental diets were cooled, packaged and stored at -20°C until use.

Proximate composition analysis of diets followed AOAC (1995) standard methods: moisture (oven-drying at 105°C), crude protein (Kjeldahl method) and crude ash (incineration at 550°C). Total lipid extraction followed Folch et al. using chloroform:methanol (v/v = 2:1). Fatty acid composition was determined according to Wu et al. by methylation with 14% boron trifluoride-methanol and analyzed using a Thermo TRACE GC ULTRA gas chromatograph equipped with an Agilent SP-2560 capillary column (100 m × 0.25 mm; 0.2 m). The temperature program was: 70°C to 140°C at 50°C/min (hold 1 min), then to 180°C at 4°C/min (hold 1 min), and finally to 225°C at 3°C/min (hold 30 min). Proximate nutrient contents and fatty acid composition of experimental diets are shown in Table 2 .

1.2 Crab Rearing Management

Experimental crabs were collected from ponds at the Chongming Base of Shanghai Ocean University. All were post-pubertal molt males weighing 135–165 g. Six hundred individuals with intact appendages, no external injuries and good vitality were selected. To better approximate production conditions, the fattening trial was conducted in outdoor small experimental earthen ponds (7.8 m × 7.8 m × 0.7 m). Double-layer anti-escape plastic panels were installed around each pond, and ponds were disinfected with bleaching powder before the trial. Pond water depth was maintained at approximately 70 cm, with rice and water peanut (*Alternanthera philoxeroides*) planted around the periphery. Each diet group had four replicates (ponds), with 25 male crabs randomly stocked per pond. After 3-5 days of acclimation, the formal experiment began on September 25, 2014.

During the trial, crabs were fed at approximately 18:00 daily. The feeding rate was 2–3% of total body weight when water temperature exceeded 20°C, and approximately 1.5% at 15–20°C. Feed was evenly distributed throughout the pond. Residual feed was checked 2–3 h after feeding, and dead crabs and residual feed around the pond were examined at approximately 09:00 the following day. Water quality parameters were measured every 3 days, and water was partially exchanged or added every two weeks based on water quality. Water temperature ranged from 11.5 to 27.2°C during the culture period, with water quality parameters as follows: pH 7.0–9.0, dissolved oxygen >4 mg/L, ammonia <0.5 mg/L, and nitrite <0.15 mg/L, all within safe ranges for Chinese mitten crab culture. The trial lasted 60 days.

1.3 Sample Collection

After 30 days of formal feeding, crabs were fasted for 1 day. Two male crabs were randomly collected from each pond (eight crabs per diet group). Surface moisture was removed with absorbent paper and body weight was measured with an electronic balance (precision = 0.01 g). Crabs were anesthetized on ice, and 2.0 mL hemolymph was extracted from the base of the third pereiopod using a 1.0 mL sterile syringe, dispensed into two 1.5 mL centrifuge tubes and stored at -40°C. The carapace was separated from the body, and hepatopancreas and gonads were dissected and weighed to calculate hepatosomatic index (HSI) and gonadosomatic index (GSI). Hepatopancreas and gonad samples were stored at -40°C for subsequent analysis.

HSI (%) = 100 × hepatopancreas weight / body weight
GSI (%) = 100 × testis weight / body weight

1.4 Sample Preparation and Lipid Metabolism Indices Determination

Approximately 0.2 g of hepatopancreas was weighed and homogenized with 1 mL (m/v = 1:5) ice-cold physiological saline using a micro-homogenizer for 30 s. Portions of the homogenate were used directly to determine lipase (LPS), lipoprotein lipase (LPL) and hepatic lipase (HL) activities. The remaining homogenate was centrifuged at 12,000 r/min for 20 min at 4°C. The middle clear layer was collected and centrifuged again, with the final supernatant used for antioxidant and immune indices analysis. Approximately 0.1 g of hepatopancreas was used for total lipid extraction following Folch et al., and the extracted lipid was dissolved in 2 mL methanol for determination of total cholesterol (TC) and triglyceride (TG) contents. Hemolymph samples were thawed, homogenized for 30 s, and centrifuged at 12,000 r/min for 20 min at 4°C. The resulting supernatant (serum) was collected for analysis.

Serum and hepatopancreas TC, TG, and serum high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) contents were measured using a Mindray automatic biochemical analyzer with commercial kits. Hepatopancreas LPL and HL activities were determined using kits from Nanjing Jiancheng Bioengineering Institute. Serum free cholesterol (FC) and hepatopancreas malate dehydrogenase (MDH) and LPS activities were measured using kits from Suzhou Keming Biotechnology Co., Ltd. Serum cholesteryl ester (CE) content was calculated as the difference between TC and FC contents.

1.5 Antioxidant Indices Determination

Using the previously prepared hepatopancreas and serum samples, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and acid phosphatase (ACP) activities, malondialdehyde (MDA) content, and total antioxidant capacity (T-AOC) were determined using kits from Nanjing Jiancheng Bioengineering Institute. Alkaline phosphatase (ALP) activity was measured using a Mindray automatic biochemical analyzer with commercial kits. Hepatopancreas -glutamyl transpeptidase (-GT) activity was determined using kits from Suzhou Keming Biotechnology Co., Ltd. Serum hemocyanin (Hc) content was measured according to Nickerson et al. by diluting serum 70-fold with Tris-Ca buffer (50 mmol/L Tris-HCl + 10 mmol/L CaCl , pH = 8.0) and measuring absorbance at 335 nm.

Hc content (mg/mL) = 3.717 × OD nm × dilution factor

1.6 Data Analysis and Statistics

Data were analyzed using SPSS 16.0 software and expressed as mean ± standard error. Levene' s test was used to check homogeneity of variance. When variance was not homogeneous, percentage data were subjected to arcsine or square root transformation. ANOVA was used for variance analysis, and Duncan' s multiple range test was used for post-hoc comparisons. Significance was set at $P < 0.05$.

2.1 Effects of Fish Oil Replacement on Gonadal Development of Adult Male Crabs

As shown in Table 3 , after 30 days of fattening, HSI ranged from 7.14% to 7.97% across groups, with diet 3# group having the highest value (7.97%) and diet 1# group the lowest (7.14%), but no significant differences were observed among groups ($P > 0.05$). Fish oil replacement by blending vegetable oils had some influence on male gonadal development, with GSI ranging from 2.16% to 2.44%. Diet 5# group showed the highest GSI, while diet 3# group had the lowest, but no significant differences were detected among groups ($P > 0.05$). After 60 days of fattening, no significant differences were found in GSI and HSI among groups ($P > 0.05$), with similar patterns to those observed at 30 days. As the fattening period progressed, HSI decreased slightly while GSI increased markedly in all groups.

2.2 Effects of Fish Oil Replacement on Lipid Metabolism of Adult Male Crabs

Hepatopancreas lipid metabolism indices are presented in Table 4 . TG content in diet 4# group was significantly lower than in other groups ($P < 0.05$), while no significant differences were observed among the remaining four groups ($P > 0.05$). No significant differences were found in TC content among groups ($P > 0.05$). Regarding lipid metabolism-related enzyme activities, MDH activity in diet 1# group was significantly higher than in diets 2# and 3# groups ($P < 0.05$), with diet 2# group showing the lowest activity. LPS activity in diet 2# group was significantly lower than in other groups ($P < 0.05$), while the remaining four groups showed increasing trends with higher fish oil replacement levels, reaching the highest value in diet 5# group. LPL and HL activities were lowest in diet 2# group, significantly lower than in diets 4# and 5# groups ($P < 0.05$), with diet 4# group showing the highest activities.

Serum lipid metabolism indices are shown in Table 5 . TG, TC and CE contents in diet 2# group were significantly higher than in the other four groups ($P < 0.05$), which showed no significant differences among themselves ($P > 0.05$). CE content in diet 2# group was significantly higher than in diet 3# group ($P < 0.05$), with no significant differences among other groups. FC content was lowest in diet 1# group, significantly lower than in diets 2# and 3# groups ($P < 0.05$), but not significantly different from diets 4# and 5# groups. LDL-C content in diet 2# group was significantly higher than in diets 1#, 3# and 4# groups ($P < 0.05$). HDL-C content was lowest in diet 5# group, followed by diet 1# group, while the remaining three groups had relatively higher values of 0.31 mmol/L.

2.3 Effects of Fish Oil Replacement on Antioxidant and Immune Capacities of Adult Male Crabs

Hepatopancreas antioxidant and immune indices are presented in Table 6 . No significant differences were observed in SOD activity among groups ($P > 0.05$). T-AOC and MDA content in diet 1# group were significantly higher than in the other four groups ($P < 0.05$), which showed no significant differences among themselves ($P > 0.05$). POD and ACP activities in diets 1# and 4# groups were significantly higher than in the other three groups ($P < 0.05$), with no significant differences among those three groups. ALP activity in diet 1# group was not significantly different from diet 5# group ($P > 0.05$) but was significantly higher than the other three groups ($P < 0.05$). -GT activity was highest in diet 4# group, significantly higher than in other groups ($P < 0.05$), with diet 2# group showing the lowest activity.

Serum antioxidant and immune indices are shown in Table 7 . SOD activity in diet 2# group was significantly higher than in other groups ($P < 0.05$). Hc content in diet 2# group was significantly higher than in other groups ($P < 0.05$), which showed no significant differences among themselves ($P > 0.05$). T-AOC in diet 2# group was significantly higher than in diets 1#, 4# and 5# groups (P

< 0.05). CAT activity was lowest in diet 3# group, followed by diet 2# group, with no significant differences among the remaining three groups ($P > 0.05$). POD activity and MDA content in diet 1# group were significantly higher than in other groups ($P < 0.05$), with diet 2# group showing the lowest values for these two indices. No significant differences were found in ACP activity among groups ($P > 0.05$). ALP activity in diet 3# group was significantly higher than in diets 1# and 4# groups ($P < 0.05$), but differences among other groups were not significant.

3.1 Effects of Fish Oil Replacement on Gonadal Development of Adult Male Crabs

The present study found that replacement of fish oil by blending vegetable oils at different levels in practical diets did not cause significant changes in male GSI, which differs from results obtained in female crabs. Studies on female crabs have shown that HUFA deficiency in fattening diets reduces ovarian index and reproductive performance. However, in this study, diets with different HUFA contents did not affect male gonadal development. This discrepancy may be attributed to different fatty acid nutritional requirements between sexes during gonadal development. Although HUFA content in male gonads is relatively high as a proportion of total fatty acids, the total lipid content and gonadosomatic index of testes are much lower than those of ovaries. Therefore, the absolute HUFA content in testes is far lower than in ovaries, suggesting that dietary HUFA requirements for male gonadal development are lower than for females. Additionally, the experimental diets used fish meal, shrimp meal and squid meal as primary protein sources, which contain certain amounts of HUFA. Consequently, even at the highest replacement level, the HUFA content in experimental diets could meet male requirements, resulting in no significant effects on gonadal development.

Liu et al. reported that high dietary n-3 HUFA [eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)] content (from fish oil, phospholipids and trash fish) significantly increased ovarian index compared with peanut oil and lard groups. This is because EPA and DHA are essential for normal ovarian development in crustaceans, and their content and ratio in ovaries directly affect spawning quantity, fertilization rate and hatching rate in some crustaceans. Therefore, different dietary HUFA contents inevitably lead to variations in female gonadal development. In contrast, male gonadal development is primarily regulated by testosterone and prostaglandins, with potentially lower dietary HUFA requirements and different mechanisms of HUFA action. Studies suggest that Chinese mitten crab may have the ability to convert linoleic acid (LOA) to arachidonic acid (ARA), which serves as a precursor for prostaglandin E2 (PGE2) and indirectly stimulates testosterone production. Hemolymph testosterone levels are positively correlated with GSI in male crabs. Furthermore, high dietary DHA content has been reported to reduce steroidogenesis in testes and decrease serum estradiol and testosterone levels in animals, possibly through n-3 PUFA effects on testicular steroidogenesis. Therefore, the mechanisms underlying differential effects of HUFA content resulting from fish oil replacement differ between sexes and warrant further investigation.

3.2 Effects of Fish Oil Replacement on Lipid Metabolism of Adult Male Crabs

The hepatopancreas is the primary organ for nutrient digestion, absorption, metabolism and storage in crustaceans, playing crucial roles in molting, immune defense and gonadal development. HSI and hepatopancreas triglyceride content are important indicators of lipid nutritional status. This study found that at days 30 and 60, both the fish oil-only group (diet 1#) and the complete vegetable oil group (diet 5#) had relatively low HSI, while the 50% replacement group (diet 3#) had relatively high HSI. This suggests that appropriate fish oil replacement levels (50%) in fattening diets benefit nutrient accumulation in the hepatopancreas, thereby increasing HSI, which may be associated with greater TG accumulation. The lowest hepatopancreas TG content was observed in the 75% replacement group (diet 4#), with no major differences among other groups, indicating that replacing 75% of fish oil with blended vegetable oils is not conducive to TG accumulation in the hepatopancreas. This may be due to altered fatty acid balance affecting lipid absorption and deposition when large amounts of fish oil are replaced. These results are similar to findings in the estuarine crab *Chasmagnathus granulata* and the edible crab *Cancer pagurus*. Some studies have shown that excessive replacement of fish oil with blended vegetable oils in juvenile turbot (*Scophthalmus maximus*) diets increased hepatic fatty acid synthase (FAS) gene expression and decreased carnitine palmitoyltransferase 1 (CPT1) expression, leading to increased total lipid content in liver and muscle. These discrepancies may be due to differences in lipid metabolism between fish and crustaceans.

MDH is closely related to lipid synthesis. In this study, MDH activities in the 25% and 50% replacement groups were significantly lower than in the fish oil-only group, suggesting that dietary n-6 PUFA inhibited MDH activity and indicating that dietary fatty acid composition affects hepatopancreas MDH activity. LPS activity in the 25% replacement group was significantly lower than in the fish oil-only group, while other groups showed significantly higher activities, similar to results in redclaw crayfish (*Cherax quadricarinatus*), suggesting that hepatopancreas LPS activity is related to dietary fatty acid composition, though this relationship requires further investigation.

LPL plays a key role in lipid metabolism regulation by catalyzing hydrolysis of lipoproteins to release free fatty acids for storage or energy supply. In this study, high-level (75% and 100%) replacement groups showed significantly higher LPL and HL activities than other groups, similar to results in gibel carp (*Carassius auratus gibelio*), but differing from studies in common carp showing decreased hepatopancreas LPL activity with increasing fish oil replacement. This may be because appropriate dietary HUFA content can promote LPL gene expression, thereby increasing hepatopancreas LPL activity. Decreased hepatopancreas LPL activity reduces fatty acid oxidation for energy, favoring lipid deposition, which may explain the relatively high TG content in diets 1#, 2# and 3# groups.

Hemolymph lipid metabolism indices can reflect overall lipid metabolism status in crustaceans. This study found that replacing 25% of fish oil with blended vegetable oils significantly increased serum TG content, suggesting this replacement level benefits TG absorption. Cholesterol serves as an important hormone precursor and membrane component in crustaceans, stored primarily as CE, and its content reflects lipid absorption and hepatic lipid metabolism status. However, crustaceans lack cholesterol synthesis capacity and must obtain it from the diet, with dietary cholesterol transported via lipoproteins in hemolymph to other tissues. In this study, the 25% replacement group showed higher serum TC and CE contents, along with higher HDL-C and LDL-C levels, indicating that replacing 25% of fish oil with blended vegetable oils benefits cholesterol absorption, transport and utilization in male crabs.

3.3 Effects of Fish Oil Replacement on Antioxidant and Immune Capacities of Adult Male Crabs

SOD is a crucial enzyme for scavenging oxygen free radicals and plays important roles in antioxidant defense and immune regulation. This study showed that replacing 25% of fish oil with blended vegetable oils significantly increased serum SOD activity, while 50–75% replacement significantly decreased it, consistent with results from juvenile crabs. T-AOC represents overall antioxidant capacity. Replacement of fish oil at different levels decreased hepatopancreas T-AOC but increased serum T-AOC, possibly because fish oil-only diets increased hepatopancreas HUFA content, requiring higher T-AOC to maintain normal physiological functions. Additionally, the fish oil-only group showed significantly higher hepatopancreas and serum POD and CAT activities, likely due to increased peroxide levels from excessive HUFA intake, prompting adaptive increases in antioxidant enzyme activities to reduce peroxide damage. MDA is a major product of lipid peroxidation. In this study, decreasing dietary fish oil (HUFA) content reduced MDA content in both hepatopancreas and serum, similar to results in juvenile crabs, suggesting that high dietary fish oil (HUFA) content may cause oxidative stress in adult crabs, resulting in higher MDA content and antioxidant enzyme activities.

Crustacean hemocyanin has important physiological functions in oxygen transport and immune defense, constituting over 90% of total hemolymph proteins. Appropriate replacement of fish oil with blended vegetable oils increased serum Hc content, possibly because proper combination of fish and vegetable oils promoted Hc synthesis, suggesting that blended oils are more beneficial than fish oil alone for oxygen transport and immunity in Chinese mitten crab. ALP and ACP are important non-specific phosphatases that catalyze hydrolysis of phosphate monoesters and phosphate group transfer reactions, playing crucial roles in immune defense. In this study, hepatopancreas ALP and ACP activities were greatly affected by dietary fish oil replacement level, while serum ALP and ACP activities were less affected. This may be because the hepatopancreas is the lipid metabolism center with high lipid content and is more susceptible to dietary lipid composition, whereas serum has lower lipid content and primarily functions in lipid transport, making its enzyme activities more stable. The results also indicated that appropriate combination of fish oil with soybean and rapeseed oils increased hepatopancreas ALP and ACP activities, suggesting that suitable fatty acid composition can improve immune capacity in aquatic animals. -GT is a plasma membrane-bound glycoprotein and a key enzyme in the -glutamyl cycle, with its activity considered a primary indicator of normal liver function. The lowest hepatopancreas -GT activity in the 25% replacement group suggests minimal oxidative stress and cellular damage. Overall, appropriate replacement of fish oil with blended vegetable oils in fattening diets can improve antioxidant and immune capacities in both hepatopancreas and serum, reduce lipid oxidation products, and decrease oxidative damage.

Conclusions:

1. Replacement of fish oil with blended vegetable oils (soybean:rapeseed oil = 1:1) in fattening diets had no significant effect on gonadal development of adult male crabs. Appropriate replacement benefited lipid absorption, transport and accumulation in the hepatopancreas, improved antioxidant and immune capacities, and reduced oxidative damage.

2. Considering gonadal development, lipid metabolism, antioxidant and immune capacities, blended vegetable oils (soybean:rapeseed oil = 1:1) can replace 25–50% of fish oil in fattening diets for adult male Chinese mitten crab.

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