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Effects of Dietary Gracilaria lemaneiformis Supplementation on Growth Performance, Serum Biochemical Indices, and Muscle Fatty Acid Composition of Grass Carp (Postprint)

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Date: 2017-10-10T00:00:00+00:00

Abstract

To investigate the effects of dietary Gracilaria supplementation on growth performance, serum biochemical indices, and muscle fatty acid composition in grass carp, six iso-nitrogenous and iso-lipidic diets containing Gracilaria at supplementation levels of 0 (control), 1%, 2%, 3%, 4%, and 5% were formulated and fed to grass carp with an average initial body weight of (15.94$±$0.10) g for 8 weeks. Each diet was assigned to 4 replicate tanks, with 30 fish per tank. The results showed: 1) Different dietary Gracilaria supplementation levels had no significant effect on weight gain rate (WGR), specific growth rate (SGR), survival rate (SR), or feed efficiency (FE) of grass carp (P>0.05). 2) The hepatosomatic index (HSI) of all treatment groups was higher than that of the control group, with significant differences observed between the control group and all treatment groups except the 5% supplementation group (P<0.05). 3) No significant differences were detected in crude protein content of whole fish, liver, or muscle among all groups (P>0.05); however, dietary Gracilaria supplementation level significantly affected crude ash and crude lipid contents of whole fish, crude lipid content of liver, and moisture content of muscle (P<0.05). 4) Serum total protein (TP), total cholesterol (TCHO), triglyceride (TG), and low-density lipoprotein (LDL) contents were lowest in the 5% supplementation group, which were significantly lower than those in the control group (P<0.05), while no significant differences were observed between other treatment groups and the control group (P>0.05). 5) Dietary Gracilaria supplementation level influenced the muscle fatty acid composition of grass carp; except for C14:1, C20:0, and C20:2, the contents of all other muscle fatty acids were significantly affected by dietary Gracilaria supplementation level (P<0.05), with muscle polyunsaturated fatty acid (PUFA) content and n-3/n-6 PUFA ratio reaching maximum values in the

3% supplementation group. The contents of muscle C20:5 (EPA), C22:5 (DPA), C22:6 (DHA), and EPA+DHA in the 1%, 2%, 3%, 4%, and 5% supplementation groups were all significantly higher than those in the control group ($P<0.05$). In conclusion, dietary supplementation with appropriate levels of Gracilaria had no significant effect on growth performance of grass carp, but could reduce blood lipid levels, alter muscle fatty acid composition, and increase the contents of functional fatty acids DHA, EPA, and PUFA; dietary supplementation with 3% Gracilaria was more effective in increasing muscle functional fatty acid content, while supplementation with 5% Gracilaria resulted in relatively better growth performance and more pronounced blood lipid-lowering effects.

Full Text

Effects of Dietary Gracilaria lemaneiformis on Growth Performance, Serum Biochemical Indices and Muscle Fatty Acid Composition of Grass Carp (Ctenopharyngodon idellus)

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Abstract

This study investigated the effects of dietary Gracilaria lemaneiformis on growth performance, serum biochemical indices, and muscle fatty acid composition of grass carp (Ctenopharyngodon idellus). Six isonitrogenous and isolipidic diets were formulated with graded levels of Gracilaria lemaneiformis at 0% (control), 1%, 2%, 3%, 4%, and 5%, and fed to grass carp with an average initial weight of (15.94$±$0.10) g for 8 weeks. Each diet was fed to four replicate tanks, with 30 fish per tank. The results showed that: (1) Different dietary inclusion levels of Gracilaria lemaneiformis had no significant effects on weight gain rate (WGR), specific growth rate (SGR), survival rate (SR), or feed efficiency (FE) ($P>0.05$). (2) The hepato-somatic index (HSI) of all experimental groups was higher than that of the control group, with significant differences observed between the control and all experimental groups except the 5% group ($P<0.05$). (3) No significant differences were found in crude protein content of whole body, liver, or muscle among groups ($P>0.05$), while significant differences were detected in whole body ash and crude lipid, liver crude lipid, and muscle moisture content ($P<0.05$). (4) Serum total protein (TP), total cholesterol (TCHO), triglycerides (TG), and low-density lipoprotein (LDL) contents were lowest in the 5% group, which were significantly lower than those in the control group ($P<0.05$), while other experimental groups showed no significant differences from the control ($P>0.05$). (5) Dietary Gracilaria lemaneiformis inclusion significantly affected muscle fatty acid composition, with all fatty acids except C14:1, C20:0, and C20:2 being significantly influenced by dietary levels ($P<0.05$). The highest values for polyunsaturated fatty acids (PUFA) content and n-3/n-6 PUFA ratio were observed in the 3% group. The contents of C20:5(EPA), C22:5(DPA), C22:6(DHA), and EPA+DHA in muscle were significantly higher in the 1%, 2%, 3%, 4%, and 5% groups compared to the control group ($P<0.05$). These results indicate that dietary supplementation with Gracilaria lemaneiformis at appropriate levels does not significantly affect growth performance but can reduce blood lipid levels, alter muscle fatty acid composition, and increase functional fatty acids such as DHA, EPA, and PUFA. A 3% dietary inclusion of Gracilaria lemaneiformis optimally increased functional fatty acid content, while 5% inclusion resulted in relatively better growth performance and more pronounced lipid-lowering effects.

Keywords: Gracilaria lemaneiformis; grass carp (Ctenopharyngodon idellus); growth performance; serum biochemical indices; muscle fatty acids

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Macroalgae are diverse and abundant, comprising four major phyla: Rhodophyta, Phaeophyta, Chlorophyta, and Cyanophyta. Together with marine phytoplankton, they constitute the primary producers in marine ecosystems. Macroalgae are rich in nutrients including polysaccharides, minerals, vitamins, free amino acids, fatty acids, natural pigments, and unknown growth factors (UGF), making them ideal ingredients for aquafeeds. The use of macroalgae as feed dates back many years, but systematic research and development of macroalgal feed additives began in the 1950s. Currently, macroalgal meal is widely used as a feed additive for livestock and poultry in developed countries such as the United Kingdom, France, and the United States, with established institutions and manufacturers dedicated to this field. In China, the application of macroalgal meal in aquafeeds is still in its infancy, with limited research reports available.

Studies have shown that macroalgae can be used as feed additives at inclusion levels of 1% to 5%, promoting animal growth, improving body coloration, and enhancing disease resistance and stress tolerance. Gracilaria lemaneiformis is a large red alga characterized by a wide temperature tolerance range [(12–23)°C], rapid growth, strong environmental adaptability, and high economic value. China possesses abundant Gracilaria lemaneiformis resources, and its development as a feed ingredient for fish could enable more scientific and effective utilization. This experiment aimed to evaluate the feasibility of using Gracilaria lemaneiformis as a feed ingredient for grass carp and to determine its optimal inclusion level by examining the effects of different dietary proportions on growth performance, body composition, and particularly muscle fatty acid composition, thereby providing guidance for developing efficient and cost-effective grass carp feeds.

1.1 Experimental Diet Preparation

Six experimental diets were formulated with wheat flour as the carbohydrate source, imported fish meal, dehulled soybean meal, and peanut bran as primary protein sources, and fish oil, soybean oil, and soybean lecithin as main lipid sources. Graded levels of Gracilaria lemaneiformis (in dry powder form, with main nutrient composition shown in Table 1) were added at 0%, 1%, 2%, 3%, 4%, and 5% to produce six diets with approximately 32% crude protein and 5% crude lipid, designated as D-1 (control), D-2, D-3, D-4, D-5, and D-6 groups. Diet composition and nutrient levels are presented in Table 2 .

All ingredients were ground to pass through a 60-mesh sieve, weighed accurately according to the formulation, and mixed stepwise for approximately 10 minutes. Soybean oil and soybean lecithin were added sequentially with continuous mixing, followed by the addition of 40% water and stirring until uniform. The mixture was then processed through a twin-screw extruder (F-26 twin-screw extruder, South China University of Technology Science and Technology Industry General Factory) and a pelletizer to produce 1.5 mm diameter pellets. The pellets were cooked in a 90°C oven for 60 minutes, then air-dried at room temperature (with air conditioning and dehumidification) to a moisture content below 10%, and stored at -20°C in sealed containers until use.

1.2 Experimental Fish and Culture Management

Experimental fish were obtained from the same batch of grass carp fry hatched in the same year. Prior to the formal experiment, the fish were acclimated in 200 L aquaria for one week and fed three times daily with a commercial grass carp feed (containing 28.80% crude protein and 3.41% crude lipid) to adapt to the experimental diets and culture conditions. After acclimation, healthy fish of uniform size [average body weight (15.94$±$0.10) g] were randomly stocked into 24 tanks, with 30 fish per tank. Each diet was fed to four replicate tanks, totaling 720 fish. During the experiment, fish were hand-fed three times daily at 09:00, 13:00, and 17:00. Every two weeks, fish in each tank were group-weighed and feed was adjusted accordingly based on body weight. The experiment lasted for 8 weeks. The culture system employed a recirculating water filtration system with regular water exchange to maintain water quality. Water temperature was maintained at 26–30°C, dissolved oxygen at 6–7 mg/L, pH at 7.0–7.4, sulfate at 0–0.05 mg/L, nitrite nitrogen at 0.05–0.10 mg/L, and ammonia nitrogen at 0.20–0.40 mg/L.

1.3 Sample Collection

At the end of the experiment, samples were collected after a 24-hour fasting period. Fish were anesthetized, and the final number and body weight of fish in each tank were recorded. Three fish per tank were randomly selected and stored at -20°C for whole-body composition analysis. Another five fish per tank were randomly selected and weighed. Blood samples were collected from the caudal vein of these fish, centrifuged at 5,000 r/min for 10 minutes to obtain serum, which was snap-frozen in liquid nitrogen and stored at -80°C until analysis. After blood collection, the liver was dissected and weighed, and dorsal muscle samples were collected and stored at -20°C.

1.4 Calculation Formulas

Weight gain rate (WGR, %) = [(final body weight - initial body weight) / initial body weight] × 100
Specific growth rate (SGR, %/d) = [(ln final mean weight - ln initial mean weight) / experimental days] × 100
Survival rate (SR, %) = (number of surviving fish / initial number of fish) × 100
Feed efficiency (FE) = (final body weight - initial body weight) / dry weight of feed fed
Hepato-somatic index (HSI, %) = (liver weight / body weight) × 100

1.5 Statistical Analysis

Experimental data are expressed as "mean ± standard deviation." Statistical analysis was performed using SPSS 11.5 software. One-way ANOVA was conducted to analyze the data, and Duncan' s multiple range test was used for post-hoc comparisons when significant differences were detected among groups. The significance level was set at $P<0.05$.

2.1 Growth and Morphological Results

As shown in Table 3 , dietary supplementation with different proportions of Gracilaria lemaneiformis had no significant effects on growth parameters (weight gain rate, specific growth rate, survival rate, and feed efficiency) ($P>0.05$). However, for morphological indices, the hepato-somatic index of all experimental groups was higher than that of the control group (D-1), with significant differences observed in all groups except D-6 ($P<0.05$).

2.2 Body Composition Results

Table 4 shows that dietary Gracilaria lemaneiformis supplementation significantly affected whole-body ash and crude lipid contents ($P<0.05$), but had no significant effects on moisture or crude protein contents ($P>0.05$). The D-6 group exhibited the highest whole-body ash content, which was significantly higher than all other groups ($P<0.05$). The D-6 group also showed the lowest whole-body crude lipid content, which was significantly lower than all groups except the control ($P<0.05$). Dietary Gracilaria lemaneiformis had no significant effects on liver moisture, ash, or crude protein contents ($P>0.05$), but significantly affected liver crude lipid content ($P<0.05$). Liver crude lipid content showed a decreasing trend with increasing Gracilaria lemaneiformis inclusion levels, with all experimental groups having lower values than the control group, and the difference between D-6 and the control group reaching significance (P<0.05). Dietary Gracilaria lemaneiformis had no significant effects on muscle ash, crude lipid, or crude protein contents (P>0.05), but significantly affected muscle moisture content (P<0.05). The D-6 group exhibited the highest muscle moisture content, which was significantly different from all other groups (P<0.05).

2.3 Serum Biochemical Indices Results

As shown in Table 5 , serum total protein (TP), total cholesterol (TCHO), triglycerides (TG), and low-density lipoprotein (LDL) contents in all experimental groups were lower than those in the control group, with the lowest values observed in the D-6 group. The D-6 group showed significant differences from the control group (P<0.05), while other experimental groups did not differ significantly from the control (P>0.05).

2.4 Muscle Fatty Acid Composition Results

Table 6 demonstrates that dietary Gracilaria lemaneiformis inclusion affected muscle fatty acid composition of grass carp. Except for C14:1 (myristoleic acid), C20:0 (arachidic acid), and C20:2 (eicosadienoic acid), the contents of all other muscle fatty acids were significantly influenced by dietary Gracilaria lemaneiformis levels (P<0.05). The contents of C20:5(EPA), C22:5(DPA), C22:6(DHA), and EPA+DHA in muscle were significantly higher in groups D-2, D-3, D-4, D-5, and D-6 compared to the control group (P<0.05), with the highest values observed in the D-4 group at (0.36$±0.03)±0.62)±0.70)±$0.72)%, respectively. Saturated fatty acids (SFA) and unsaturated fatty acids (UFA) contents reached maximum values in D-3 and D-5 groups, respectively, while monounsaturated fatty acids (MUFA) content was lowest in the D-4 group. Polyunsaturated fatty acids (PUFA) content was highest in the D-4 group. Dietary Gracilaria lemaneiformis inclusion also significantly affected n-3 PUFA, n-6 PUFA contents, and n-3/n-6 PUFA ratio (P<0.05), with the highest n-3/n-6 PUFA ratio observed in the D-4 group, which was significantly higher than all groups except D-3 (P<0.05).

3.1 Effects of Dietary Gracilaria lemaneiformis on Growth, Morphology, and Body Composition of Grass Carp

The present results indicate that dietary Gracilaria lemaneiformis supplementation had no significant effect on grass carp growth. While few studies have reported on Gracilaria lemaneiformis as an aquafeed additive, numerous studies have examined the effects of other macroalgae on fish growth. Zhou et al. found that compared with the control group, the growth performance of spotted rabbitfish (Siganus oramin) was not significantly affected by 5% dietary Enteromorpha inclusion, but was significantly reduced at 10% and 15% inclusion levels. Optimal growth was achieved in Japanese flounder (Paralichthys olivaceus) fed diets containing 3% Eucheuma muricatum. Dietary inclusion of 4% Ulva lactuca improved growth in gilthead sea bream (Sparus aurata). These examples demonstrate that low concentrations of macroalgae can promote fish growth, while high concentrations are detrimental. This may be attributed to two factors: first, macroalgae are rich in carbohydrates, polysaccharides, and cellulose, which can interfere with protein and dry matter utilization; second, macroalgae contain antinutritional factors that hinder growth at high inclusion levels. In this study, no significant differences in growth were observed among groups. Although growth tended to decrease with Gracilaria lemaneiformis inclusion up to 4%, the highest weight gain and specific growth rates were observed at 5% inclusion, which differs from previous findings. This discrepancy may be due to species-specific, macroalgae-specific, and dose-dependent effects, warranting further investigation.

The hepato-somatic index of grass carp in experimental groups (1%–5% inclusion) was higher than that of the control group, with the maximum value observed in the D-4 group and the minimum in the D-6 group. Güroy et al. reported that diets containing 10% Ulva reduced the hepato-somatic index in rainbow trout (Oncorhynchus mykiss), while Azaza et al. observed similar reductions in Nile tilapia (Tilapia nilotica) fed diets with different Ulva inclusion levels, attributing this effect to reduced hepatic lipid deposition. In the present study, although liver crude lipid content in all experimental groups was lower than in the control group (Table 4), the hepato-somatic index was higher, contradicting previous findings. This may be explained by the fact that the liver serves not only as an important energy storage organ but also as a primary site of intermediary metabolism, and its weight can change significantly with nutritional status. Yang et al. suggested that the liver, as a crucial immune organ, exhibits a higher organ index (ratio of immune organ to body weight) as an indicator of faster maturation. The increased hepato-somatic index in experimental groups may reflect accelerated liver maturation with Gracilaria lemaneiformis supplementation, while the reduced index in the D-6 group may be due to the dominant lipid-reducing effect of 5% macroalgae inclusion and the relatively larger body weight of fish in this group.

3.2 Effects of Dietary Gracilaria lemaneiformis on Serum Biochemical Indices of Grass Carp

Dietary Gracilaria lemaneiformis inclusion significantly affected serum biochemical indices in grass carp. Changes in blood TP content can reflect hepatic synthetic dysfunction and protein loss due to kidney pathology, serving as an indicator of fish response to environmental stressors. Blood TCHO content reflects whole-body lipid metabolism, with 70%–80% of cholesterol originating from the liver and a small amount from the digestive tract; serum cholesterol levels change when hepatocellular dysfunction or damage occurs. LDL transports endogenous cholesterol and cholesterol esters throughout the body, while blood TG content is an important physiological indicator of lipid metabolism in fish, with elevated levels indicating excessive fat accumulation in the liver and increased risk of fatty liver and hepatomegaly. Matanjun et al. found that feeding mice a mixture of three macroalgae at 5% inclusion reduced serum TG and TCHO levels. Sun et al. reported that dietary Enteromorpha inclusion at 4% significantly reduced serum TG and TCHO levels in broiler chickens after 6 weeks. These findings are consistent with our results. Macroalgae can reduce blood glucose and lipid levels through two mechanisms: first, they contain natural bioactive substances with hypoglycemic and hypolipidemic properties. Nakajima found that dietary S-dimethyl-β-propiothetin (a bioactive compound in macroalgae) reduced blood cholesterol and TG levels. Second, the high n-3 PUFA content in macroalgae also exerts lipid-lowering effects. In this study, serum TP, TCHO, TG, and LDL levels in all experimental groups were lower than in the control group, with the lowest values observed in the D-6 group, which differed significantly from other groups. Overall, dietary Gracilaria lemaneiformis supplementation can reduce blood lipid levels and promote liver health in grass carp, with the D-6 group showing superior performance based on these indices.

3.3 Effects of Dietary Gracilaria lemaneiformis on Muscle Fatty Acid Composition of Grass Carp

All experimental groups fed Gracilaria lemaneiformis-supplemented diets exhibited significantly higher muscle DHA, EPA, DHA+EPA, PUFA, n-3 PUFA contents, and n-3/n-6 PUFA ratio compared to the control group.

Humans cannot synthesize C20:4 [arachidonic acid (AA)] (not detected in grass carp muscle), EPA, C18:3 [linolenic acid (ALA)], or DHA. The primary dietary sources of EPA and DHA for inland populations are fish and shrimp. Most studies on macroalgae supplementation and muscle fatty acid composition have focused on livestock and poultry, with limited research on aquatic animals. Tang found that dietary macroalgae rich in PUFA could alter milk fatty acid composition, significantly increasing functional fatty acids such as EPA and DHA. Franklin et al. reported that feeding dairy cows approximately 4% Schizochytrium sp. resulted in milk fat DHA content much higher than in the control group, corroborating our findings. Yi et al. demonstrated that DHA and EPA contents in large yellow croaker (Larimichthys crocea) muscle decreased with reduced dietary DHA and EPA, but the rate of decrease was less than 1, indicating that DHA and EPA tend to accumulate in large yellow croaker to maintain normal physiological functions. Similar conclusions were drawn in studies on Atlantic salmon (Salmo salar) and rainbow trout. Dawczynski et al. found that macroalgae are rich in n-3 PUFA, n-6 PUFA, EPA, and ALA, and that macroalgal feeds can provide essential fatty acids for fish muscle. Additionally, Dantagnan et al. reported that macroalgae contain natural bioactive substances that can affect fatty acid metabolism, and the synergistic effect of these compounds can enrich these fatty acids in fish muscle. The higher DHA and EPA contents in muscle of experimental groups in this study may be attributed to the high DHA and EPA content in Gracilaria lemaneiformis. High levels of UFA, particularly PUFA, in the diet help prevent cardiovascular and cerebrovascular diseases. It has been documented that Greenland Eskimos, who consume large quantities of marine products, have significantly elevated blood n-3 PUFA levels. Xu et al. fed spotted rabbitfish with four different diets including Enteromorpha, Gracilaria lemaneiformis, fresh fish, and formulated feed, and found that macroalgae groups had significantly lower whole-body crude lipid content but higher muscle PUFA content than the fresh fish and formulated feed groups. In our study, all experimental groups had higher muscle PUFA content than the control group, consistent with these findings. The n-3/n-6 PUFA ratio is also important. Simopoulos suggested that although the n-3/n-6 PUFA ratio may vary with health status, a higher ratio may be more effective in reducing the risk of many chronic diseases. Gogus et al. found that dietary n-3/n-6 PUFA ratio was negatively correlated with various diseases. In this study, all experimental groups fed Gracilaria lemaneiformis had higher muscle n-3/n-6 PUFA ratios than the control group, with the maximum value observed in the D-4 group, indicating that grass carp fed macroalgae-supplemented diets may be healthier for human consumption.

Conclusions: 1. Dietary inclusion of 1%–5% Gracilaria lemaneiformis had no significant effect on grass carp growth performance. 2. A 3% dietary inclusion of Gracilaria lemaneiformis improved muscle fatty acid composition and increased beneficial fatty acid content, while 5% inclusion resulted in better growth performance and serum biochemical indices. 3. Overall, dietary Gracilaria lemaneiformis supplementation reduced blood lipid levels and improved fatty acid composition in grass carp, better meeting human nutritional needs.

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