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Feeding effects of low-level fish meal replacement by algal meals of *Schizochytrium limacinum* and *Nannochloropsis salina* on largemouth bass (*Micropterus salmoides*)

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Abstract

Microalgae exhibit diverse and high nutritional value, and therefore, have a huge potential application as a raw material for the preparation of aquafeeds. This study was conducted to evaluate the effects of low-level replacement of dietary fishmeals by algal meals comprised of *Schizochytrium limacinum* (SL) and *Nannochloropsis salina* (NS) individually and in combination in the diet of juvenile largemouth bass (LMB, *Micropterus salmoides*). Five isonitrogenous, isolipidic, and isoenergetic diets with fishmeal-based diets as control, and four experimental diets with replacement by individual 4% SL (4SL), individual 4% NS (4NS), 2% SL combined with 2% NS (2SL+2NS) and 4% SL combined with 4% NS (4SL+4NS) were formulated. Five hundred juvenile fish (average weight 8.72 ± 0.10 g) were evenly distributed to five groups with quadruplicate each, in twenty fiberglass tanks and fed for eight weeks. Our results showed that there were no significant difference in weight gain, special gain rate, hepatosomatic index, viscerosomatic index, conditioning factor, and the whole-body composition among the five groups ($p > 0.05$). However, feed intake and feed conversion ratio in the 4NS treatment were significantly higher ($p < 0.05$). compared with the control. Further, lipid content and intraperitoneal fat ratio in the liver of the 4NS and 4NS+4SL groups were significantly decreased, suggesting that NS may reduce visceral fat accumulation in LMB. The serum superoxide dismutase (SOD) activity of LMB in the 4NS group was significantly increased compared with the control ($p < 0.05$). However, malondialdehyde (MDA) content in the liver of the 4NS group and intestine of the 4SL+4NS group was significantly lower than that in the control ($p < 0.05$). Replacement with 4% SL significantly increased docosahexaenoic acid, polyunsaturated fatty acids (PUFAs), and n-3 PUFA proportions in the liver. Further, SL alone or in combination with NS increased the n-3 to n-6 ratio of PUFAs in the liver and muscle, creating more balanced unsaturated fatty acids. The fillet texture was significantly enhanced by taste and flavor. Nevertheless, the present study verified the successful addition of SL and NS algal meals to fish feed, which exhibited excellent potential to replace fishmeals and fishoil, respectively, as protein and lipid sources.

Keywords: Algal meal; feed replacement; growth performance; fatty acids; *Micropterus salmoides*

1. Introduction

With the rapid increase in aquaculture consumption and production in China, the demand for marine-based ingredients, such as fishmeals (FM) and fish oil (FO), has also risen. Approximately 70% of global supplies of FM and FO are used by farmed fish (Subhash et al., 2020). Huge consumption of these commodities leads to overexploitation of marine resources, a decrease in human food security, and an increase in the prices of FM and FO (Sarker et al., 2018). Investigating suitable alternatives to FM and FO without affecting fish growth and health is an urgent need in aquaculture (Sarker et al., 2016). Among all the potential alternatives, algae have been recognized as promising ingredients in aquafeeds due to their high protein and fatty acid contents, and their results in similar or better growth performance, when compared to FM and FO in various aquatic species (Allen et al., 2019; Seong et al., 2020). Algal meals are also rich in bioactive compounds such as vitamins, minerals, pigments, and antioxidants, which promote the health of aquatic animals through improving their biological functions, including antioxidant, immunomodulatory, and antibacterial properties (Chen et al., 2019a). Compared with other terrestrial plant ingredients (e.g., soybean), algae are rich in essential fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which cannot be synthesized by animals in vivo. Thus, many types of algae have proven to be a useful component of the diet to increase growth and improve physiological activity, disease resistance, stress response, and fillet texture (Adissin et al., 2019; Ayala et al., 2020). In addition, ingested algae may also stimulate the synthesis of endogenous enzymes and provide exogenous enzymes that benefit digestion (Mélo et al., 2016). Therefore, as substitutes for FM and FO in aquaculture, algae have many advantages and have attracted great interest from the scientific community and aquaculture industries (Osmond et al., 2021).

Among various algal species, *Schizochytrium limacinum* (SL) is produced through heterotrophic reproduction and is considered to be a microorganism with great potential for the industrial production of DHA (Osmond et al., 2021; Sarker et al., 2016). It has been experimentally confirmed that replacing FO in Pacific white shrimp (*Litopenaeus vannamei*) with SL significantly improved antioxidant activities and induced no negative effect on muscle lipid peroxidation (Allen et al., 2019). Previous studies have demonstrated that partial or complete replacement of dietary FO with *Schizochytrium* sp. oil had no negative effects on

the digestibility of dietary proximate nutrients in juvenile Atlantic salmon (Tibbetts et al., 2020). *Schizochytrium* meal feeding was found to improve the growth performance or total long-chain polyunsaturated fatty acids in golden pompano (*Trachinotus ovatus*) and giant grouper *Epinephelus lanceolatus* (Xie et al., 2019, García-Ortega et al., 2016).

Nannochloropsis salina (NS) is a unicellular microalga with a polysaccharide cell wall and one chloroplast. Previously, It was reported that animals have a good tolerance to NS oil without any negative effects (Chen et al., 2012; Gbadamosi et al., 2018). Compared with traditional oleaginous crops, NS has a high potential yield with the elevated n-3 PUFA and amino acid content, and even the potential yield of NS is appreciable, compared with traditional oleaginous crops (Gbadamosi et al., 2018; Qiao et al., 2019). The addition of NS to aquafeeds increased the growth and survival of seahorse juveniles (*Hippocampus reidi*) (Mélo et al., 2016). Valente et al. (2019) demonstrated that the replacement FM level of defatted *Nannochloropsis sp*. meal could reach 15% without affecting the proximate composition and growth performance of fish. Therefore, numerous research results have proven that SL and NS as substitutes for FM and FO in the feed are advantageous to fish, which will help to promote the healthy and rapid development of the aquaculture industry. The combination of multiple algae meals may provide additional benefits, making full use of each meal to achieve excellent results for fish growth (Ma et al., 2014). However, replacing FM and FO with the more expensive algal meals is not economically suitable at present. Therefore, finding a low-level algal replacement that achieves the above-mentioned at a low cost is the main target of this study.

Micropterus salmoides, usually called largemouth bass (LMB), is a freshwater fish native to North America. It has been introduced to China and has become a very popular cultural species, especially in Guangdong Province (Habte-Tsion et al., 2020; Wu et al., 2021). The annual production in China has been maintained at approximately 200,000 tons, of which Guangdong Province is the main breeding ground for LMB, accounting for more than 60% of the total domestic production (Jiang et al., 2018). As a carnivorous fish, LMB has a high requirement for fishmeals (approximately more than 45%) in commercial feed (Han et al., 2018). Full or partial replacement with algal meals will decrease dependence on fishmeals during LMB breeding. Previous studies suggested that small addition or replacement of algal

increased growth performance, improved appetite, immune response, or mucle DHA (Luo et al., 2018; Xie et al., 2019; Chen et al., 2019b; Santos et al., 2019). However, the digestibility of nutrients from microalgae has been suggested to be influenced by their intrinsic cell wall structure and indigestible non-starch. Tibbetts et al. (2020) proposed that the inclusion of dry whole-cell *Schizochytrium* meal in farmed Atlantic salmon and rainbow trout should be limited to a very low level (10%). Based on the immune, antioxidant, anti-infammatory, and lipid metabolism capacities, previous research indicated that *Schizochytrium* extracted oil could completely replace FO in the LMB diet, but this study did not evaluate the replacement of FM by *Schizochytrium* and associated impact on fish growth (Hebte-Tsion et al., 2020). To the best of our knowledge, the effects of SL and NS meals on the growth of LMB have not been experimentally investigated yet. Therefore, the objective of this study was to assess the impact of low-level dietary substitution of FM and FO with algal meals of *Schizochytrium limacinum* (SL) and *Nannochloropsis salina* (NS), individually and in combination on growth performance, serum biochemical indices, enzyme activity, fatty acid composition and fillet texture, and finally to determine the feasibility of these algae as feed ingredients for carnivorous fish such as LBM *Micropterus salmoides*. Additionally, from an economic point of view, this study aims to look for a low-level substitution that will achieve the goal of ensuring fish health growth and enhancing meat quality.

2. Materials and Methods

2.1 Ethical statement

All the fish used in this study were acclimated under standard laboratory conditions and had access to standard water and food requirements (SYXK-2013-100). All procedures were conducted in accordance with the “Guiding Principles in the Care and Use of Animals(China)” and were approved by the Laboratory Animal Ethics Committee of the Guangdong Academy of Agricultural Science, Guangzhou, China (2020G010). Through humane husbandry management techniques and optimization measures, we tried our best to reduce fish suffering and ensure fish survival.

2.2 Diet preparation

SL and NS whole cell dry powders were provided by SDIC Biotech Investment Co., Ltd. (Beijing, China). The proximate composition was determined, as shown in Table S1(Supplementary material). Five isonitrogenous, isolipidic and isoenergetic diets (Table 1) were formulated to contain 48% crude protein and 11.8% crude lipid (dry weight). Five diets with different percentages of SL and NS dry weight were formulated as follows: nonalgal replacement as Control (Con), 4% SL (4SL), 4% NS (4NS), 2% SL combined with 2% NS (2SL+2NS) and 4% SL combined with 4% NS (4SL+4NS), which was relevant to 0~7.8% of fishmeals（Table S2）. The FM, FO and flour content were adjusted to level protein, lipids and the total weight of the diets (Table 1). All the raw material for the diets was weighed according to the feed formula, ground and mixed evenly with water. Then extruded into stripes at suitable rotational speed by a twin-screw extruder (SLX-80, Guangdong Academy of Agricultural Sciences) and automatically cut to pellets with a particle size of 1.5 mm. The diet pellets were dried in an oven at 55 ℃ for approximately five hours and sieved through 2 mm sieves. The approximate nutritional composition was determined and analyzed to confirm that the five diets were isonitrogenous, isolipidic and isoenergetic. Then, diets were stored in airtight containers in a -20 ℃ refrigerator to reduce the oxidization of fatty acids until needed.

2.3 Experimental fish

Juvenile LMB fish were obtained from Liang's Aquatic Seed Industry Company (Guangdong, China) and were acclimated and fed with a basal diet (Con) for two weeks in the Aquatic Laboratory, South China Agriculture University (Guangdong, China). Following the ethical guidelines for fish treatment, five hundred healthy juvenile LMB (with an average weight of 8.72 ± 0.10 g) were selected and evenly distributed to five groups with quadruplicate each, in twenty experimental fiberglass tanks (350 L per tank) containing 300 L running water in a closed recirculating aquaculture system (Fig. S1) and continuous aeration with oxygenation pumps (810-7AH07). Each diet was fed to fish regularly at 9: 00 and 17: 00 every day to apparent satiety and food intake was recorded. The feeding experiment lasted for eight weeks with water quality parameters of dissolved oxygen above 6.20 mg/L, water temperature 25.8-27.2 °C, and pH 6.85-7.35 (determined by YSI Professional Plus

multiparameter instrument, Xylem Inc., USA). Total ammonia nitrogen (Nessler's reagent colorimetric method, Purkinje General, model T6, Beijing) and nitrite (α -naphthylamine method, Purkinje General, model T6, Beijing) in the recirculating aquaculture system were measured from time to time to ensure that their concentration remained below 0.2 mg/L.

2.4 Sampling

At the end of the eight-week experiment, the fish were sampled after 24 hours of fasting. The total number and weight of the fish in each tank were recorded. For the whole-body composition analysis, three fish from each tank were randomly selected, weighed, and stored at -20 ℃. Another three fish were anesthetized (Pentobarbitone 1:10,000), weighed and dissected to collect visceral mass, liver, intraperitoneal fat samples. The collected tissue samples were weighed and stored at -20 ℃. One milliliter (1 mL) of blood sample was collected by syringe and injected into a 1.5 mL EP (Eppendorf) tube. Serum was extracted by centrifugation of blood samples at 4000 r/min for ten minutes at 4 ℃, after standing samples at room temperature for four hours. Back muscle on both sides was collected for fillet texture determination and then stored at -20 ℃ for the subsequent determination of muscle composition and fatty acids.

2.5 Growth performance

Fish and the diets fed to each tank were weighed, and the fish biometric parameters were calculated using the following formula:

(1) Food intake (FI, % BW/day) = Grams of dry feed consumed × 100 × 2 / [56× (Initial fish weight + Final fish weight + Dead fish body weight)]

(2) Weight gain (WG, %) = [(Final fish weight - Initial fish weight) / Initial fish weight] × 100.

(3) Specific growth rate (SGR, %/day) = 100 × [Ln (final fish weight) - Ln (initial fish weight)] / 56 days.

(4) Feed conversion ratio (FCR) = Feed intake (g) / (Final fish weight - Initial fish weight) (g).

(5) Survival rate (SR, %) = (Final fish number / Initial fish number) ×100.

(6) Condition factor (CF, %) = [Fish body weight / (Length of fish) ^{3}] × 100.

(7) Hepatosomatic index (HSI, %) = (Weight of liver / Fish body weight) × 100.

(8) Viscerasomatic index (VSI, %) = (Weight of viscera / Fish body weight) × 100.

(9) Intraperitioneal fat ratio (IPF, %) = (Weight of intraperitioneal fat / Fish body weight) × 100.

2.6 Proximate composition analysis

Samples of diets, whole fish and liver were freeze-dried to constant weight, grounded and mixed for homogeneity. Moisture (oven drying at 105 °C to constant weight), crude protein (Kjeldahl's method, semiautomatic Kjeldahl System, Buchi, Flawil, Switzerland), crude lipid (extraction with petroleum ether, Soxhlet extractor, VELP Scientific, Milano, Italy) and ash (combustion using a Thermolyne™ muffle furnace at 600 °C for four hours) of the samples were performed following the standard procedures in GB/T 14924.9-2001. The gross energy content of the experimental diets was calculated on the basis of the thermal energy for protein, lipids, and glycogen/starch.

2.7 Biochemical indices and enzymatic activities analysis

The main serum parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acid (TBA), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were determined with a blood analyzer using diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China). Enzymatic activities, such as lipase (LPS), superoxide dismutase (SOD) and malondialdehyde (MDA), in the intestine, liver and serum were also determined through diagnostic reagent kits (Nanjing Jiancheng Bioengineering Institute, China). One unit of lipase activity was defined as per g or mL of protein or serum reacted with the substrate for 1 minute in the reaction system and consumed 1 μmol of the substrate at 37 ℃. One unit of SOD was defined as the content of SOD per mL of reaction solution when the SOD inhibition rate reached 50%. The amount of MDA was defined as per g or mL of protein or serum reacted with the substrate in the reaction system. All indices were measured three times by the same operator to ensure the accuracy of the data.

2.8 Fatty acids composition analysis

The fatty acid composition in two algae, five diets (Table 2), the liver and muscle was

determined by the boron trifluoride methylation method. Firstly, the freeze-dried sample was soaked in the chloroform-methanol solution for 48 hours to extract fatty acids from the sample. Subsequently, boron trifluoride was used as a catalyst to thoroughly methylate the extracted fatty acids. The methyl ester sample was extracted with n-hexane and separated by gas chromatography (GC, Agilent Technologies, 7890B). For GC analysis, nitrogen was used as the carrier gas at a pressure of 300 kPa and hydrogen was used as a fuel gas. The retention time of various fatty acid absorption peaks in the sample was determined by comparison with appropriate fatty acid methyl ester standards. Finally, the composition of the corresponding fatty acids in the sample was determined, and the concentration of individual fatty acids was expressed as a percentage of the total content.

2.9 Fillet texture

The back muscles were taken from the back starting point parallel to the outer side of the pectoral fin base point. The muscle samples were examined by a cylindrical probe with a Universal TA Texture Analyzer (Tenglon Instrument Company, Shanghai, China) to determine their brittleness, adhesiveness, springiness, chewiness, gumminess, cohesiveness, and resilience. Then, the cylindrical probe was changed to the shearing knife to determine the shearing force (Yu et al., 2020). Every index was determined five times on average. A force of one kg was evenly applied to the muscle to determine the water retention. The muscle was boiled for five minutes to determine the cooked meat rate.

2.10 Statistics analyses

The data were generally presented as means ± SE. The differences in values among the five groups were analyzed by one-way analysis of variance (ANOVA) using the SPSS 25.0 software package (SPSS Inc.). Tukey's test was used for multiple comparison analyses. A statistical significance level of $p = 0.05$ was employed. The figures were created and retouched with GraphPad Prism 8 and Adobe Illustrator 2018.

3. Results

3.1 Growth assessment

Throughout the trial, survival was 99-100% for fish consuming all experimental diets.

(Table 3**)**. There were no significant differences in WG, SGR HSI, VSI, CF and the whole body proximate composition among the five groups ($p > 0.05$). Compared with the control group, FI and FCR in the 4NS treatment group were increased by 10.5% and 12.2%, respectively ($p < .05$). IPF in the three groups with NS was significantly lower than in the groups without NS ($p < 0.05$). The crude protein in the liver of 4SL+4NS groups was slightly higher than that of the other groups ($p < 0.05$). The crude lipids in the liver of the 4NS and 4SL+4NS groups was observed at 2.00% and 2.22%, which was significantly lower than other groups, and the muscle crude lipids content of the 4NS group were significantly lower than other groups ($p < 0.05$).

3.2 Biochemical indices and enzymatic activities

After ingestion of SL and NS diets by the fish, the impacts on serum biochemical indices and responsese of enzymatic activities are highlighted in Fig. 1. The serum ALT and ALP activities in the experimental group did not show significant differences compared with the control ($p > 0.05$), but AST activity in the 4NS and 2NS+2SL was significantly lower than that in the control ($p < 0.05$). The mixed algal supply had significantly lower ALP activity than the individual supply. There was no significant difference in TBA and HDL contents among the five groups ($p > 0.05$). However, LDL in the 4NS group was increased by 32.3%, compared to the control group ($p < 0.05$). As for the liver, when compared with the control, the MDA concentration in the 4NS group was decreased by 24.8% ($p < 0.05$), and the LPS activity of the 4NS and 4SL+4NS was increased by 10.3% and 9.57% ($p < 0.05$). However, in the intestine, the MDA content in the 4SL+4NS was significantly reduced ($p < 0.05$). The SOD activity in the liver and intestine showed no significance among the five groups ($p > 0.05$).

3.3 Fatty acids profile

The fatty acid profiles of the liver and muscle are presented in Tables 4 and 5. The fatty acid profiles of the LMB significantly changed after ingestion of SL and NS algal meals, both diets presenting different fatty acid profiles. In the liver, the levels of SFA (C14:0; C20:0; C21:0) and MUFA (C15:1, C16:1; C18:1n-9; C18:1n-11; C24:1n-9) in the 4SL group were significantly decreased ($p < 0.05$) while those of PUFA (C18:2n-6, C22:2n-6; C22:6n-3) were

significantly increased by 26.5%, compared to the control groups ($p < 0.05$). Among evaluated fatty acids, the EPA (C20:5n-3) content impacted less, while the DHA (C22:6n-3) content in the 4SL group was enhanced by 11.3%, approximately increased twice compared to the control group (3.50%). At the same time, the n-3 to n-6 ratio of PUFAs was significantly increased ($p < 0.05$). As for muscles, the n-3 PUFAs and total PUFAs in the 4NS group were significantly lower than those of the other four groups ($p < 0.05$). And, the n-3 to n-6 ratio of PUFAs was significantly decreased in the 4SL+4NS group ($p < 0.05$).

3.4 Fillet texture

The results of the fillet texture of LMB are shown in Table 6. The chewiness in the 2SL+ 2NS group was significantly higher than that of the other four groups ($p < 0.05$), and the gumminess in the 4NS group was significantly lower than that of the other four groups ($p < 0.05$). However, the water retention in the 4SL group was significantly higher than that of the 4NS and 4SL+4NS groups ($p < 0.05$). There were no negative impacts on the shearing force, brittleness, adhesiveness, springiness, cohesiveness, resilience, or cooked meat rate of the fillet.

4 Discussion

The nutritional value of an ingredient for fish species depends on its chemical composition and the bioavailability of its nutrients and energy to the fish, which can be evaluated by growth indices and body composition (Kousoulaki et al., 2016; Batista et al., 2020). The results obtained in the present work revealed that 2% or 4% *Schizochytrium limacinum* (SL) and *Nannochloropsis salina* (NS) replacement individually and in combination with fishmeals and fish oil had no negative effect on growth performance in LMB juveniles. Many other studies indicated similar results when the replacement was relatively smaller. Inclusion of 5~6% *Schizochytrium* sp. in Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*), as partial substitution of fishmeals, had no negative effects on growth performance (Kousoulaki et al., 2016; Lyons et al., 2017). However, a higher level substitution of *Schizochytrium* sp. in the diet may reduce the growth of Atlantic salmon (Kousoulaki et al., 2016). Dietary inclusion of *Nannochloropsis* sp. up to 10% did not hamper the growth of juvenile turbot (*Scophthalmus maximus* L.) and *Salmo*

salar (Qiao et al., 2019; Gong et al., 2020), but when the inclusion increased up to 20%, Atlantic salmon growth decreased (Sørensen *et al.*, 2016). In other studies, it was found that the addition of extra low levels (3%) of *Schizochytrium* sp. increased the growth of golden pompano (*Trachinotus ovatus*) (Xie et al., 2019). When completely replacing fish or soybean meals, *Oreochromis niloticus* fed *Nannochloropsis salina* showed comparable growth performance to soybean meals but a significantly lower growth rate than fishmeals (Gbadamosi et al., 2018). Algal meals composed of *Arthrospira* sp. and *Schizochytrium* can successfully replace 10~50% of fishmeals and soy protein concentration for hybrid striped bass and red drum *Sciaenops ocellatus* (Perez-Velazquez et al., 2018; Perez-Velazquez et al., 2019). These results suggest that the response of fish growth performance to the amount of algae supplied depends on fish species and the specific formula of the diet. Further research is needed to determine the effects of a high supplemental level of algae meals on the growth of LBM.

In the present study, the special growth rate of LMB ranged from 3.64~3.74 % / day, which was higher than those recently reported by Zhao et al. (2021), i.e., 2.6% / day, Rahman et al. (2021), i.e., 1.98~2.32%, and Xin et al. (2020), i.e., 2.37~2.45 % / day, employing smaller or larger fish sizes. These findings highlighted the relatively good growth performance of the fish in the present study. Compared with the control, 4% NS replacement significantly increased the feed intake, indicating good palatability of NS, which was also found in Atlantic salmon (*Salmo salar*) that received 20% *Nannochloropsis oceania* (Sørensen et al., 2016). However, 14% mixed biomass of *Nannochloropsis* sp. and *Isochrysis* sp. decreased Atlantic cod (*Gadus morhua*) juvenile feed intake (Walker et al., 2011). Compared with the control, the reduced feed conversion ratio in the 4NS group indicated a relatively lower utilization of NS for LMS. Therefore, to balance the energy of digesting NS, more food needs to be consumed, thereby increasing the FI and FCR. Previous studies have shown that cell wall disruption can increase nutrient accessibility from microalgae (Agboola et al., 2019; Teuling et al., 2019). Investigating methods to disrupt the algal cell wall will be more beneficial to fish. Biometric parameters, including HIS, VSI and CF of LMB, were not affected by the algal supply. However, the IPF in the three groups containing NS was significantly higher than that in the non-NS groups. Low-level substitution of fishmeals by

NS and SL had no significant effect on whole body composition, which is similar to the results of various algae diets as replacements for hybrid striped bass (de Cruz et al., 2018). The liver lipid content in the 4NS and 4NS+4SL groups and the muscle lipid content in the 4NS group were significantly reduced, which was in accordance with IPF, suggesting that NS may reduce visceral fat accumulation in LMB. Similarly, other research has suggested that dietary *Schizochytrium* sp. to *Salmo salar* and *Aurantiochytrium* to juvenile yellowtail (*Seriola quinqueradiata*) could reduce the lipid content in the liver (Kousoulaki et al., 2016; Fukada et al., 2019). However, *Schizochytrium* did not show the same effects as NS in the present study.

Serum biochemical indices of aquatic animals are widely used to evaluate the health, nutrition and adaptation of fish to the environment and are excellent indices for physiological and pathological evaluation (Benli et al., 2004). Serum ALT, AST and ALP are important indicators of liver function. When hepatocytes are damaged by inflammation or necrosis, they are released into the bloodstream, causing increased activity (Souza et al., 2020). In the present study, compared with the control, the algal replacement groups did not show any significant change in the activities of ALT and ALP, but AST activity significantly decreased in the 4NS and 2NS+2SL groups. These results suggest that the algal replacement generally has no negative effects on liver function. LPS activity is an indicator of lipid digestion ability. Compared with the control, the activity of LPS in the serum and liver of both the 4NS and 4NS+4SL groups was significantly increased, which was also proven by the lower liver content and IPF ratio mentioned above.

Algae are considered a natural source of antioxidants. It is widely known that algae have a variety of effects on the fish's antioxidant system (Castro et al. 2020). For instance, 5% *Nannochloropsis* sp. replacement of FM in turbot (*Scophthalmus maximus*) diets enhanced the fish antioxidant capacity by increasing serum and liver SOD activity (Qiao et al., 2019). However, the replacement of 20% dietary FM with *Nannochloropsis oceania* dried meals did not affect serum SOD activity in Atlantic salmon (Sorensen et al., 2017). When fishmeals were replaced by *Arthrospira platensis*, the serum SOD activity of gibel carp (*Carassius auratus gibelio* var. CAS III) was not significantly changed, albeit, after bacterial challenge, the algal supplementation led to a significant increase in the plasma SOD level in gibel carp.

In the present study, the serum SOD activity of LMB in the 4NS group was significantly increased compared with the control group, but SOD activity in the liver and intestine was not significantly different among the five groups. Further, MDA content in the liver of the 4NS group and the intestine of the 4SL+4NS group was significantly lower than that in the control group. These results indicated that NS could enhance the antioxidant capacity of fish, finally resulting in decreasing MDA concentrations in the liver and intestine, as it was also found that dietary *Nannochloropsis* sp. significantly reduced the MDA content of juvenile turbot (*Scophthalmus maximus*) serum and liver (Qiao et al., 2019).

Docosahexaenoics (DHA, C22:6n-3) and eicosapentaenoics (EPA, C20:5n-3), belong to the n-3 LC-PUFAs, are essential nutrients for all vertebrates, including fish (Ganuza et al., 2008; Eryalçın; et al., 2013; Yadav et al., 2020) and humans (Osmond et al., 2021). Fish are the main sources of DHA and EPA for humans, while the fatty acid composition of fish tissue is closely related to the diet (Gong et al., 2020). It has been reported that *Schizochytrium sp.* has potential as an alternative DHA source for fish (Ganuza et al., 2008; Miller, et al., 2007; García-Ortega et al., 2016). In the present study, after the replacement of SL and NS, the prepared diets comprised more than 40% MUFA and 30% PUFA, with the n-3 to n-6 PUFA ratio close to 0.2-0.4. At the end of the experiment, a significantly higher DHA proportion was observed in the liver of the 4SL group, reflecting a higher proportion of DHA and PUFAs in *Schizochytrium* sp. compared with fishmeals and *Nannochloropsis* sp. It was also suggested that SL was well digested and absorbed and can effectively protect the digestive organs of the fish, such as the liver (Perez-Velazquez et al., 2018). A significantly lower DHA proportion in the fish muscle in the 4NS group may indicate insufficient DHA levels in *Nannochloropsis* sp. However, previously, when 10% *Nannochloropsis oceanica* was used to replace 50% fishmeals and 10% fish oil, the whole-body EPA and DHA levels of Atlantic salmon remained similar to those of the control group (Gong et al., 2020). This study further suggested that the PUFA level of the algae was not only determined by algae species but also attributed to algae culturing conditions. Low levels of algal meal replacement not only ensured that there are no negative effects on the fish, but also improved fish fatty acids to a certain extent, providing ideas and directions for the future development of fishery feeds. However, the undetectable levels in muscle and a much lower proportion of EPA in the fish

liver were observed in all the groups from the present study, contrary to the previous studies (Gbadamosi et al., 2020; Gong et al., 2020; Perez-Velazquez et al., 2018). Wu et al. (2002) indicated that EPA was less important than DHA because the fish requirements for EPA may be minimal or EPA could be reconverted from DHA. The good growth performance of LMB in the present study, as mentioned above, indicated that fish growth was not affected by very low EPA level. Despite the level of PUFAs, the balance of the n-3 to n-6 PUFA ratio is also important for human mental health, and a lower n-3 to n-6 PUFA ratio may increase inflammation, accelerating illnesses, such as heart diseases (Young et al., 2009; Simopoulos et al., 2011). In this study, the liver n-3 to n-6 PUFA ratio in groups containing algae meals was significantly increased, compared with the control ($p < 0.05$). Due to the high level of DHA in SL, the n-3 to n-6 PUFA ratio in the liver and muscle of fish supplied with SL was higher ($p < 0.05$). Furthermore, SL combined with NS decreased the n-6 PUFAs in muscle at the same time, resulting in a significant increase in the n-3 to n-6 ratio of PUFAs, compared with the 4NS group ($p < 0.05$). In conclusion, considering the low level of algae replacement, SL alone or combined with NS could decrease the n-3 to n-6 ratio of PUFAs in the liver, as well as in the muscles. Perhaps this is an effective way to provide more balanced unsaturated fatty acids for humans.

Fillet texture is an important indicator for evaluating flavor when it is eaten, which affects the fish value (Yu, et al., 2020). Water retention is one of the important characteristics of muscle mass and reflects the ability to retain fluid and soluble substances in the muscle, as loss of water will result in the loss of water-soluble proteins, soluble aroma substances and hemoglobin in the muscle, reducing fillet texture (Yang et al., 2022). Lower chewiness and gumminess and higher water retention suggest a good flavor of the fish (Lv 2015). In the present study, it was found that the replacement of 4% NS had the lowest chewiness and gumminess although the significance level did not reach except compared with the 2SL+2NS group. The 4NS group had higher water retention, although the significance level did not reach either. In summary, supplementation with the 4NS tended to improve the flavor of LMB.

5. Conclusion

This study showed that low levels of dietary fishmeals (FM) and fish oil (FO) replacement by algal meals of *Schizochytrium limacinum* (SL) and *Nannochloropsis salina* (NS) individually and in combination in the diet had no negative effect on the growth performance or the whole body composition of juvenile largemouth bass (LBM) (*Micropterus salmoides*). Supplementation of low-level NS either individually or in combination with SL can significantly decrease the liver lipid content and intraperitoneal fat ratio and possibly increase the liver and intestine antioxidant capacity. Supplementation with low-level SL can increase the liver DHA proportion and the n-3 to n-6 ratio of PUFAs. Supplementation with 4% NS may improve the flavor of LMB. Therefore, SL and NS meals can be promising low level substitutes for fishmeals, which provide improved fish quality as healthier food.

CrediT authorship contribution statement

Hongping, Liao: Conceptualization, Research performance, Methodology, Formal analysis, Data curation, Writing - original draft; Huijuan, Tang: Conceptualization, Methodology, Formal analysis, Writing - review, Funding acquisition; Peiqin, Liu, Yongyan, Deng, Wenqi, Zhang, Ciguang Pan, & Youming Jia: Conceptualization, Research performance.

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables

Table 1 Diets formulation and composition of experimental diets fed to LMB.

Content (%)	Con	4SL	4NS	2SL+2NS	4SL+4NS
Fish meals	60.0	57.9	57.4	57.6	55.3
Soybean meals	15.0	15.0	15.0	15.0	15.0
Wheat flour	13.0	12.0	12.4	12.3	11.6
Fish oil	5.00	4.18	3.95	4.07	3.14
Soybean oil	3.00	3.00	3.00	3.00	3.00
Schizochytrium sp. meals	0.00	4.00	0.00	2.00	4.00
Nannochloropsis sp. meals	0.00	0.00	4.00	2.00	4.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00	1.00	1.00
Choline Chloride	0.50	0.50	0.50	0.50	0.50
Monocalcium phosphate	1.50	1.50	1.50	1.50	1.50
Analyzed composition					
Moisture (%)	6.35	6.90	6.65	6.35	7.00
Crude protein	48.4	48.3	48.4	48.4	48.3
Crude lipid	11.8	11.8	12.1	11.8	11.8
Ash (%)	12.5	10.7	10.4	11.3	12.0
Gross energy (MJ/kg)	18.0	18.0	18.1	18.0	18.0

Schizochytrium sp. meals (Crude protein: 35.9%, crude lipid: 23.4%) and *Nannochloropsis* sp. meals (Crude protein: 43.5%, crude lipid: 29.8%) (We determined them in laboratory of South China Agricultural University).

Fish meals (Crude protein: 67.0%, crude lipid: 5.60%), soybean meals (Crude protein: 44.2%, crude lipid: 1.90%), wheat flour (Crude protein: 12.4%, crude lipid: 1.30%), fish oil, soybean oil, choline chloride, monocalcium phosphate (Provided by Guangzhou Fishtech Biotechnology Co., LTD).

Vitamin premix provided the following per kg of diets: VA 48 mg, VD 24 mg, VE 80 mg, V K_3 20 mg, VB_1 20mg, VB_2 40mg, VB_6 24 mg, VB_{12} 0.08 mg, niacin 140 mg, folic acid 6.425 mg, inositol 200 mg, calcium pantothenate 80 mg, biotin 0.32 mg, moisture ≤ 10% (Provided by Guangzhou Fishtech Biotechnology Co., LTD).

Mineral premix provided the following per kg of deits: K 180 mg，Ca 1150 mg，Mg 45 mg，Zn 40 mg，Fe 50 mg，Mn 9.5 mg，Co 1.25 mg，Se 0.25 mg，I 0.16 mg，moisture ≤ 10% (Provided by Guangzhou Fishtech Biotechnology Co., LTD).

Table 2 Fatty acids composition of experimental diets and SL and NS meal.

Content (%)	Con	4SL	4NS	2SL+2NS	4SL+4NS	SL	NS
C14:0	4.01	4.09	3.93	4.51	4.91	3.21	2.95
C14:1	0.05	0.08	0.09	0.01	0.02	3.72	0.25
C15:0	0.27	0.53	0.37	0.49	0.61	0.00	0.00
C15:1	15.35	17.61	17.61	18.04	19.76	45.3	22.0
C16:0	0.18	0.19	0.21	0.23	0.26	0.12	0.03
C16:1	4.14	4.12	5.42	4.82	6.09	0.15	29.8
C17:0	0.33	0.45	0.38	0.44	0.50	1.54	0.18
C17:1	0.59	0.54	0.40	0.73	0.54	0.03	0.07
C18:0	3.81	3.88	5.33	3.98	3.72	0.06	0.04
C18:1n-9	15.7	14.5	15.9	14.1	13.1	0.28	5.54
C18:1n-11	4.00	3.35	3.13	3.28	3.08	0.21	0.87
C18:2n-6	22.2	18.5	18.5	17.1	14.3	0.58	3.60
C20:0	0.12	0.10	0.12	0.09	0.07	0.10	0.44
C18:3n-6	0.04	0.09	0.03	0.08	0.05	0.00	0.12
C20:1	4.38	3.82	3.38	3.42	3.47	0.21	0.02
C21:0	1.21	1.15	1.41	1.44	1.45	0.16	0.04
C20:2n-6	0.47	0.45	0.53	0.57	0.53	0.00	0.07
C22:0	0.21	0.12	0.25	0.18	0.22	0.11	0.58
C20:3n-6	0.20	0.43	0.27	0.16	0.24	0.11	0.00
C22:1n-9	0.91	0.87	0.97	0.92	1.06	0.15	2.38
C20:3n-3	0.73	0.86	0.86	0.86	0.99	0.00	0.00
C23:0	0.20	0.14	0.19	0.13	0.06	0.19	0.04
C20:4n-6	0.35	0.59	0.33	0.30	0.44	0.41	0.07
C22:2n-6	7.68	7.64	7.45	7.31	8.70	0.35	25.4
C20:5n-3	0.26	0.21	0.37	0.17	0.28	0.09	0.00
C24:1n-9	0.11	0.18	0.12	0.16	0.24	0.03	0.09
C22:5n-3	0.19	0.63	0.27	0.46	0.66	6.83	0.11
C22:6n-3	6.57	8.79	5.65	6.91	8.40	31.8	0.04
SFA	10.3	10.7	12.2	11.5	11.8	5.58	4.37
MUFA	45.3	45.1	47.0	45.5	47.3	50.1	60.9
PUFA	38.7	38.3	34.3	34.0	34.6	40.2	29.4
n-3 PUFA	7.75	10.5	7.15	8.40	10.3	38.7	0.15
n-6 PUFA	31.0	27.8	27.2	25.6	24.3	1.44	29.2
n-3/n-6 PUFA	0.25	0.38	0.26	0.33	0.42	26.9	0.01

SFA (Saturated fatty acids), MUFA (Monounsaturated fatty acids), PUFA (Polyunsaturated fatty acids)

Table 3 Growth performance, biometric parameters and body proximate composition of LMB fed with experimental diets (n = 4).

Growth performance	Con	4SL	4NS	2SL+2NS	4SL+4NS
IBW (g)	8.72±0.00	8.72±0.01	8.72±0.01	8.73±0.01	8.73±0.01
FBW (g)	70.2±2.98	70.8±2.68	66.3±0.59	70.1±3.33	67.4±5.00
FCR (g/g)	0.82±0.04^{a}	0.89±0.04^{ab}	0.92±0.06^{b}	0.88±0.05^{ab}	0.90±0.01^{ab}
FI (%/d)	2.28±0.08^{a}	2.49±0.10^{ab}	2.52±0.16^{b}	2.45±0.11^{ab}	2.47±0.07^{ab}
WG (%)	714±41.3	696±32.2	667±11.6	695±38.3	672±58.1
SGR (%/d)	3.74±0.09	3.70±0.07	3.64±0.03	3.70±0.09	3.65±0.14
SR (%)	100±0.00	99.0±2.00	100±0.00	99.0±2.00	100±0.00
Biometric parameters index					
HSI (%)	1.26±0.20	1.21±0.17	1.23±0.22	1.24±0.13	1.21±0.18
VSI (%)	7.16±0.52	6.95±1.02	6.44±0.16	6.47±0.20	6.28±0.31
CF (g/cm^{3})	2.25±0.20	2.27±0.28	2.12±0.09	2.17±0.07	2.14±0.11
IPF (%)	2.84±0.25^{b}	2.65±0.79^{b}	1.43±0.09^{a}	1.64±0.21^{a}	1.32±0.19^{a}
Whole body					
Moisture (%)	74.3±2.17	74.5±0.78	75.1±0.09	74.2±1.00	74.7±0.78
Crude protein (%)	15.9±0.70	15.5±0.25	15.6±0.59	16.1±0.55	16.0±0.72
Crude lipid (%)	6.47±2.48	6.95±0.73	6.40±0.27	6.75±1.69	5.44±0.69
Ash (%)	4.71±0.88	5.00±0.20	5.67±0.76	5.69±0.24	5.49±0.90
Liver composition					
Moisture (%)	78.5±1.72	77.4±0.76	78.6±1.21	76.1±1.08	78.0±4.50
Crude protein (%)	9.13±0.53^{a}	9.62±0.52^{a}	8.77±0.56^{a}	9.62±0.38^{a}	11.0±1.26^{b}
Crude lipid (%)	3.07±0.54^{c}	2.73±0.20^{bc}	2.00±0.17^{a}	3.14±0.44^{c}	2.22±0.41^{ab}
Ash (%)	1.10±0.25	1.32±0.12	1.42±0.21	1.10±0.14	1.27±0.36
Muscle composition					
Moisture (%)	81.6±0.50	80.9±0.67	80.5±0.30	80.6±0.85	80.5±0.78
Crude protein (%)	15.1±0.75	16.2±0.74	16.7±0.53	16.5±0.70	15.8±0.80
Crude lipid (%)	1.91±0.26^{b}	1.88±0.41^{b}	1.12^{a}±0.19	1.93±0.24^{b}	1.80±0.28^{b}
Ash (%)	0.95±0.11	0.88±0.08	1.02±0.09	0.72±0.42	1.00±0.19

Data are presented as mean ± S.E. Values with different superscripts in the same row are significantly different (p < 0.05). The lack of superscript letter indicates no significant differences among groups (p > 0.05).

IBW (Initial body weight); FBW (Final body weight); FCR (Feed conversion ratio); FI (Feed intake); WG (Weight gain); SGR (Special gain rate); SR (Survival rate); HSI (Hepatosomatic index); VSI (Viscerasomatic index); IPF (Intraperitioneal fat ratio); CF (Condition factor).

Table 4 Fatty acids composition of LMB liver fed with experimental diets (n = 4).

Liver	Con	4SL	4NS	2SL+2NS	4SL+4NS
C14:0	3.86±0.23^{b}	2.70±0.33^{a}	4.02±0.10^{b}	4.17±0.09^{b}	3.86±0.22^{b}
C14:1	0.42±0.03	0.46±0.07	0.53±0.10	0.59±0.03	0.52±0.14
C15:1	26.8±2.40^{ab}	23.9±2.82^{a}	28.0±2.60^{ab}	29.1±1.00^{ab}	30.9±2.83^{b}
C16:0	0.38±0.05	0.38±0.06	0.43±0.02	0.47±0.03	0.39±0.02
C16:1	4.42±0.28^{b}	3.22±0.27^{a}	4.71±0.18^{b}	4.44±0.11^{b}	4.36±0.12^{b}
C17:0	0.69±0.02	0.67±0.09	0.68±0.06	0.80±0.02	0.84±0.15
C17:1	0.13±0.04	0.16±0.05	0.15±0.08	0.13±0.03	0.12±0.01
C18:0	11.4±1.17	10.9±1.00	10.6±0.56	11.8±1.35	11.8±0.36
C18:1n-9	20.8±1.33^{b}	16.4±0.74^{a}	21.4±2.88^{b}	19.7±1.59^{ab}	18.1±0.69^{ab}
C18:1n-11	5.19±1.27^{a}	4.54±1.12^{a}	8.25±1.59^{b}	4.35±0.47^{a}	5.88±0.86^{ab}
C18:2n-6	6.67±2.16^{a}	11.3±1.39^{b}	6.52±1.30^{a}	5.74±1.59^{a}	8.75±3.82^{ab}
C20:0	0.23±0.04^{ab}	0.10±0.04^{a}	0.16±0.12^{ab}	0.24±0.02^{b}	0.21±0.05^{ab}
C20:1	1.64±0.32^{ab}	2.00±0.18^{b}	1.50±0.12^{ab}	1.62±0.16^{ab}	1.27±0.14^{a}
C21:0	0.73±0.05^{b}	0.36±0.06^{a}	0.71±0.17^{b}	0.85±0.13^{b}	0.83±0.07^{b}
C20:2n-6	0.70±0.04	0.82±0.03	0.72±0.06	0.77±0.12	0.68±0.03
C22:0	0.19±0.10	0.29±0.16	0.16±0.12	0.15±0.08	0.20±0.04
C22:1n-9	0.24±0.06	0.18±0.09	0.18±0.08	0.25±0.02	0.23±0.05
C20:3n-3	0.70±0.12^{ab}	1.25±0.21^{b}	0.72±0.51^{ab}	0.83±0.09^{ab}	0.50±0.33^{a}
C23:0	0.75±0.38	0.31±0.04	0.80±0.22	1.33±0.86	0.52±0.12
C22:2n-6	0.64±0.05^{a}	1.61±0.51^{b}	0.64±0.09^{a}	0.50±0.14^{a}	0.53±0.16^{a}
C20:5n-3	0.27±0.15	0.22±0.04	0.26±0.02	0.22±0.13	0.18±0.07
C24:1n-9	0.66±0.15^{b}	0.09±0.05^{a}	0.75±0.06^{b}	0.82±0.08^{b}	0.85±0.08^{b}
C22:6n-3	3.50±0.16^{a}	11.3±3.93^{b}	4.06±0.93^{a}	3.26±0.81^{a}	3.01±0.63^{a}
SFA	18.5±0.58^{ab}	15.7±1.70^{a}	17.5±2.20^{ab}	20.1±1.00^{b}	20.3±1.59^{b}
MUFA	63.4±2.75^{b}	51.2±5.06^{a}	65.9±1.30^{b}	62.5±4.03^{b}	62.1±5.01^{b}
PUFA	14.2±2.43^{a}	26.5±5.65^{b}	12.5±1.75^{a}	12.6±0.36^{a}	11.4±3.08^{a}
n-3	3.75±0.49^{a}	12.7±4.04^{b}	4.57±0.79^{a}	4.68±0.44^{a}	4.31±0.54^{a}
n-6	10.5±2.46^{bc}	13.8±1.75^{c}	7.88±1.43^{ab}	7.02±1.75^{ab}	5.59±0.72^{a}
n-3/n-6 PUFA	0.32±0.03^{a}	0.92±0.19^{c}	0.59±0.15^{b}	0.58±0.07^{b}	0.77±0.04^{bc}

Data are presented as mean ± S.E. Values with different superscripts in the same row are significantly different (p < 0.05). The lack of superscript letter indicates no significant differences among groups (p > 0.05).

Table 5 Fatty acids composition of LMB muscle fed with experimental diets (n = 4).

Muscle	Con	4SL	4NS	2SL+2NS	4SL+4NS
C14:0	2.71±0.38^{ab}	2.91±0.40^{ab}	2.28±0.46^{a}	3.53±0.05^{b}	3.00±0.35^{ab}
C14:1	0.19±0.06	0.18±0.03	0.17±0.04	0.18±0.05	0.21±0.04
C15:0	0.27±0.04^{a}	0.41±0.03^{b}	0.21±0.07^{a}	0.43±0.04^{b}	0.45±0.07^{b}
C15:1	19.0±0.88^{b}	18.7±1.76^{b}	9.86±1.67^{a}	20.2±1.76^{b}	19.4±1.74^{b}
C16:0	0.20±0.05	0.24±0.04	0.19±0.06	0.26±0.06	0.24±0.02
C16:1	3.55±0.36	3.52±0.72	2.84±1.12	4.13±0.09	4.07±0.84
C17:0	0.39±0.11	0.40±0.02	0.31±0.11	1.44±2.15	0.37±0.04
C17:1	0.31±0.08	0.37±0.17	1.14±1.56	0.37±0.04	2.08±1.98
C18:0	5.16±1.00	4.29±0.66	3.25±1.32	4.48±0.53	4.57±1.44
C18:1n-9	16.4±0.46	16.2±0.82	18.0±4.71	16.5±0.88	14.0±1.76
C18:1n-11	3.76±0.62^{a}	3.51±1.21^{a}	8.78±0.20^{b}	4.05±1.53^{a}	6.18±0.91^{ab}
C18:2n-6	15.7±2.68	15.0±1.07	11.7±3.68	16.1±1.07	12.5±2.19
C18:3n-6	0.17±0.09	0.21±0.03	0.15±0.03	0.14±0.08	0.18±0.04
C20:1	2.64±0.43^{b}	2.50±0.61^{b}	1.48±0.03^{a}	3.07±0.20^{b}	2.31±0.28^{ab}
C21:0	0.48±0.08	0.46±0.11	0.35±0.11	0.52±0.14	0.63±0.23
C20:2n-6	0.75±0.14^{ab}	0.71±0.03^{ab}	0.48±0.16^{a}	0.88±0.15^{b}	0.78±0.06^{ab}
C22:0	0.44±0.12	0.35±0.08	0.26±0.12	0.39±0.04	0.28±0.06
C22:1n-9	0.33±0.08	0.47±0.09	0.28±0.12	0.43±0.17	0.34±0.08
C20:3n-3	0.65±0.14^{ab}	0.89±0.16^{b}	0.46±0.13^{a}	0.67±0.21^{ab}	0.84±0.06^{ab}
C20:4n-6	0.28±0.05^{ab}	0.38±0.05^{b}	0.17±0.04^{a}	0.26±0.08^{ab}	0.29±0.06^{ab}
C22:2n-6	2.22±0.28^{ab}	2.81±0.39^{a}	1.67±0.34^{a}	2.85±0.36^{a}	2.78±0.20^{a}
C22:5n-3	0.46±0.08^{a}	0.75±0.13^{b}	0.32±0.16^{a}	0.73±0.09^{b}	0.79±0.13^{b}
C22:6n-3	11.6±1.39^{b}	13.2±0.91^{b}	6.17±0.70^{a}	12.0±1.27^{b}	12.5±2.45^{b}
SFA	10.2±0.75	9.07±1.26	11.5±6.84	11.5±2.34	9.27±2.02
MUFA	46.5±1.68	46.8±2.67	56.0±9.91	48.7±2.14	46.7±5.02
PUFA	33.5±2.28^{b}	34.1±2.44^{b}	19.7±3.29^{a}	33.3±1.62^{b}	31.0±5.30^{b}
n-3 PUFA	12.6±1.19^{b}	14.9±1.03^{b}	6.78±0.91^{a}	13.3±1.26^{b}	14.2±2.74^{b}
n-6 PUFA	19.4±3.12	19.2±1.45	14.6±4.33	20.0±1.37	16.8±2.60
n-3/n-6 PUFA	0.56±0.02^{a}	0.78±0.03^{bc}	0.65±0.09^{ab}	0.69±0.05^{b}	0.84±0.05^{cd}

Data are presented as mean ± S.E. Values with different superscripts in the same row are significantly different (p < 0.05). The lack of superscript letter indicates no significant differences among groups (p > 0.05).

Table 6 Fillet texture of LMB fed with experimental diets (n = 4).

	Con	4SL	4NS	2SL+2NS	4SL+4NS
Shearing force (gf)	964±82.6	1096±157	1014±90.	1221±220	964±151
Brittleness (gf)	300±10.0	302±35.4	299±43.6	332±19.0	298±34.6
Adhesiveness (gf)	-2.39±0.10	-2.22±0.55	-2.03±0.2	-2.26±0.12	-2.36±0.20
Springiness (gf)	0.31±0.02	0.29±0.03	0.26±0.01	0.31±0.03	0.28±0.01
Chewiness (gf)	34.3±6.02^{a}	34.0±12.1^{a}	24.7±1.00	40.0±5.88^{b}	30.7±4.44^{a}
Gumminess (gf)	96.6±23.8^{a}	108±19.7^{a}	89.1±4.54	116±9.60^{b}	104±11.8^{ab}
Cohesiveness (gf)	0.34±0.05	0.36±0.03	0.34±0.04	0.36±0.04	0.34±0.02
Resilience (gf)	0.39±0.05	0.37±0.04	0.39±0.05	0.37±0.03	0.36±0.04
Water retention (%)	8.57±1.72^{a}	6.91±1.31^{a}	13.4±3.68	10.6±2.80^{a}	10.7±2.64^{b}
Cooked meat rate (%)	16.1±5.86	15.9±2.62	19.4±2.56	20.8±3.94	21.4±1.94

Data are presented as mean ± S.E. Values with different superscripts in the same row are significantly different (p < 0.05). The lack of superscript letter indicates no significant differences among groups (p > 0.05).

Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

Declaration of competing interest

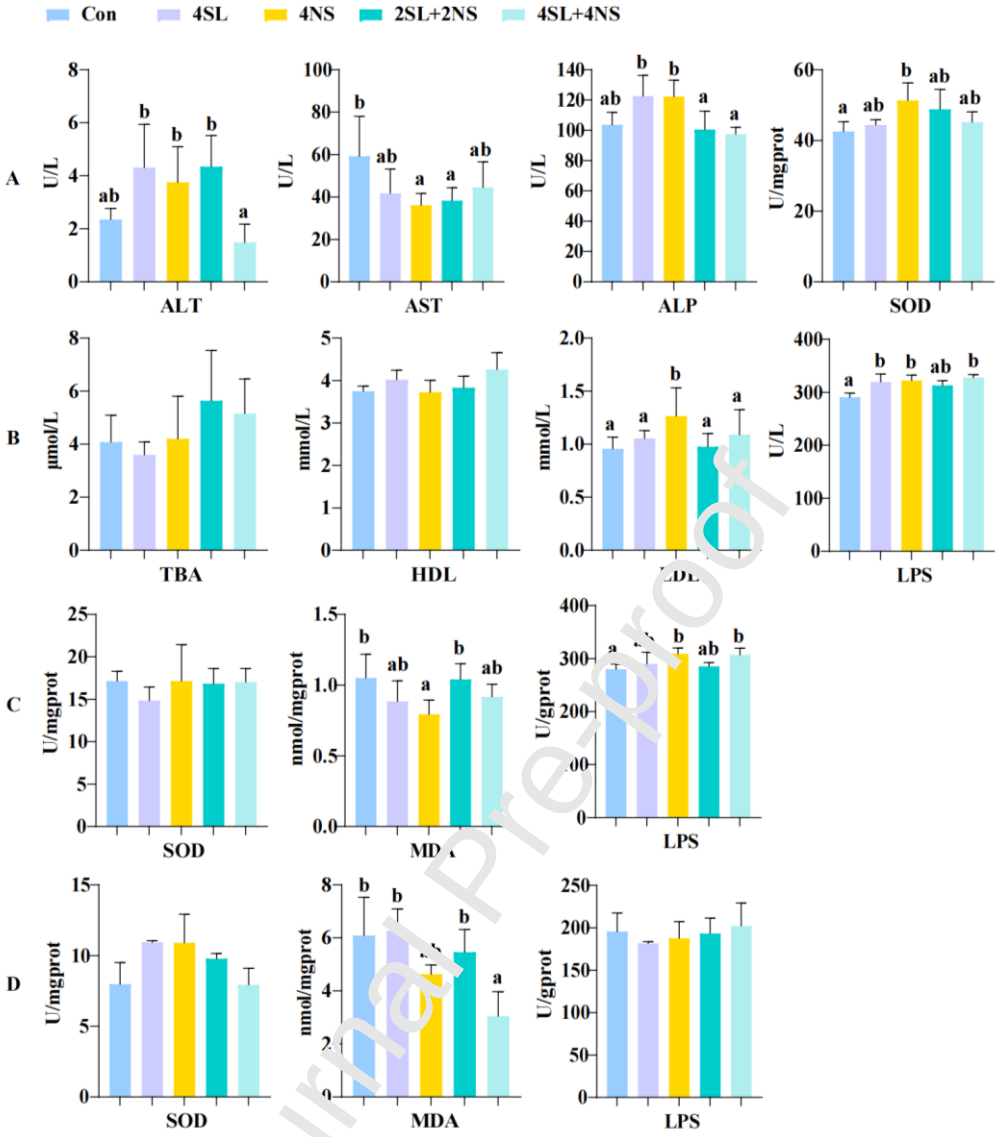
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Figure captions

Fig. 1 Serum biochemical indices and histology enzyme activity of LMB fed with experimental diets (n = 4).

A, B (serum biochemical indices and enzyme activity), C (enzyme activity of liver), D (enzyme activity of intestine). Column with different superscripts are significantly different ($p < 0.05$). The lack of superscript letter indicates no significant differences among groups ($p > 0.05$).

TBA (Total bile acid); GLU (Glucose); HDL (High density lipoprotein); LDL (Low density lipoprotein); ALT (Alanine aminotransferase); AST (Aspartate aminotransferase); ALP (Alkaline phosphatase); SOD (Superoxide dismutase); LPS (Lipase); MDA (Malondialdehyde).



Highlights

- Effects of fishmeal replacement by algal meals on largemouth bass were evaluated.
- Low-level substitution had no negative effect on growth or whole-body composition.
- Fishmeal replacement (4%) with *Nannochloropsis salina* decreased lipid deposition.
- Fishmeal replacement (4%) with *Schizochytrium limacinum* increased docosahexaenoic acid.