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Research Article

Effects of Formulated Diet and Frozen Fresh Fish on Growth, Serum Biochemical Indexes, Liver Antioxidant, and Lipid Metabolism of Juvenile Cobia (*Rachycentron canadum*)

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Feed is the main source of material and energy for farmed fish, and its nutritional value and balance are important factors affecting fish's growth rate and physical health. In order to explore the effects of two different feed sources on the growth, serum biochemical indexes, liver antioxidant capacity, and lipid metabolism of young cobia fish, 300 young cobia fish with an initial body weight of 43.14 ± 1.25 g were selected for the experiment and randomly divided into two treatments. Each treatment had five replicates, and each replicate had 30 fish. They were fed with formulated feed and frozen fresh fish, respectively, for 12 weeks. The results showed that the weight gain rate, specific growth rate, and feed conversion rate of juvenile cobia fish fed with formulated diet were extremely significantly lower than those of the frozen fresh fish group ($P<0.01$), and condition factor was significantly lower than that of the frozen fresh fish group ($P<0.05$). However, protein efficiency rate, hepatosomatic index, and viscerosomatic index were significantly higher than those of frozen fresh fish group ($P<0.05$). The content of water, crude protein, and crude ash in the whole fish had no significant difference ($P>0.05$), while the content of crude lipid decreased significantly ($P<0.05$). The serum aspartate aminotransferase activity and sugar content of cobia in the formulated diet group were significantly higher than those in the frozen fresh fish group ($P<0.05$). In contrast, the total cholesterol, triglyceride content, alkaline phosphatase activity, and phosphorus content were significantly lower than those in the frozen fresh fish group ($P<0.05$). Compared with the frozen fresh fish group, the antioxidant enzyme activities in the liver of the formulated diet group were significantly decreased ($P<0.05$) except malondialdehyde (MDA) and the activity of fatty acid synthase, while there was no significant difference in the malate dehydrogenase. The study showed that under the experimental conditions, frozen fresh fish was more suitable for feeding juvenile cobia, and the formulated diet had adverse effects on the liver of juvenile cobia. Therefore, the nutritional composition of frozen fresh fish and the metabolic characteristics of cobia were used for reference to optimize and adjust the nutritional formula of juvenile cobia.

1. Introduction

As the only extant member of the genus Rachycentron and the family Rachycentridae, cobia (*Rachycentron canadum*) is a species of carangiform marine fish. *Rachycentron canadum*, commonly known as cobia, is a highly valued marine fish species with significant commercial importance in the aquaculture [1–4]. As an integral part of the Chinese southern coast marine cage culture industry, it plays a vital role [5]. Currently, the feed for the cultivation of cobia in China is still mainly frozen fresh miscellaneous fish. At the same time, the formulated diet is only used in a small amount in the

cultivation process due to its high cost and poor breeding efficiency. With the continuous expansion of the scale of aquaculture, the aquaculture mode based on feeding fresh and miscellaneous fish still needs to meet the needs of intensive aquaculture. In aquaculture feed, fish meal (FM) has been identified as the most preferred animal-origin protein component as a result of its valuable properties [6–8]. FM is derived from baitfish for large-scale fish, which is important for human consumption [6]. For carnivorous species especially, high-protein content plays a significant role in fish nutrition in terms of balanced amino acid and fatty acid content and high digestibility [7]. Due to the fact that FM is produced from fish obtained by fishing, production quantities fluctuate. FM, the main protein source in feeds, is becoming more expensive, as the demand for feeds increases. Aquaculture's sustainable growth can be adversely affected by the use of FM as a protein source [6–8]. In order to meet the growing demand for cobia, efforts are been made to develop efficient feeding strategies that enhance growth, improve nutritional quality, and optimize the health status of the fish. One important aspect of cobia rearing is the evaluation of different diets and their impact on the growth performance, serum biochemical indexes, liver antioxidant capacity, and lipid metabolism.

There are some disadvantages of frozen miscellaneous fish, such as the sharp reduction of resources, difficulty in storage, easy to decay, bacteria breeding, and aquaculture water eutrophication. On the other hand, due to the unique characteristics of cobia, such as its living habits and physiological mechanism, there are high requirements for nutrition, crushing granularity, mixing uniformity, feed formability, and water resistance of cobia feed, and the existing cobia feed is difficult to meet these requirements. Therefore, how to optimize the formula of formula feed, improve the nutritional ratio of feed, and replace the frozen fresh miscellaneous fish has become a research focus. In recent years, studies on the nutritional requirements of cobia mainly focus on the protein [9–11], lipid [12–14], carbohydrate [15, 16], minerals [17] etc. Therefore, a full understanding of the nutritional requirements of cobia is the prerequisite for the scientific and reasonable preparation of its artificial feed. Formulated diets have gained popularity in aquaculture due to their cost-effectiveness and ease of preparation, but their nutritional composition and impact on fish health need to be evaluated against natural food sources such as frozen fresh fish.

Growth performance is a key parameter to assess the efficacy of a specific diet, and it includes measurements such as weight gain, feed conversion ratio, and specific growth rate (SGR) [9, 15, 16]. Serum biochemical indexes, including blood glucose, total protein, albumin, triglycerides, and cholesterol levels, are reliable indicators of the nutritional status and metabolic health of fish [2, 3]. The liver plays a vital role in antioxidant defense mechanisms and lipid metabolism [18, 19]; hence, the evaluation of liver antioxidant enzyme activities and lipid profiles provides insights into the overall health and well-being of cobia. Comparative studies analyzing the effects of formulated diets and frozen fresh fish on cobia are essential for sustainable aquaculture practices. By understanding the impact of different feeding strategies on growth, serum biochemical indexes, liver antioxidant capacity, and lipid metabolism, we can optimize diet formulation and feeding protocols to enhance cobia production, improve fish health, and minimize the environmental impact of aquaculture operations.

The primary objective of this study was to investigate the effects of a formulated diet compared to frozen fresh fish on the growth performance, serum biochemical indexes, liver antioxidant capacity, and lipid metabolism of juvenile cobia.

Table 1: Composition of the basal diet (dry weight) %.

Items	Content
Fish meal	41.00
Peeled soybean meal	19.00
Wheat flour	32.82
Corn oil	1.00
Soybean lecithin	1.00
Fish oil	2.00
Vitamin C (35%)	0.05
Choline chloride	0.50
Vitamin premix[1]	0.20
Mineral premix[2]	0.20
Cellulose	0.50
Attractant	0.10
Ethoxy quinoline	0.03
$Ca(H_2PO_4)_2{\cdot}H_2O$	1.60
Total	100.00

Notes. [1]The vitamin premix provided the following per kg of the diet: VA 12 000 IU, VD 4 000 IU, VE 400 mg, VK_3 40 mg, VB_1 60 mg, VB_2 200 mg, VB_6 40 mg, VB_{12} 0.4 mg, calcium pantothenate 280 mg, nicotinic acid 800 mg, biotin 6 mg, folic acid 15 mg, inositol 400 mg, PABA 400 mg, β-carotene 12 mg. [2]The mineral premix provided the following per kg of the diet: KCl 953.47 mg, $MgSO_4{\cdot}7H_2O$ 1 024.44 mg, ferric citrate 850.25 mg, $ZnSO_4{\cdot}7H_2O$ 353.57 mg, $CuSO_4{\cdot}5H_2O$59.84 mg, $MnSO_4{\cdot}H_2O$ 96.26 mg, $CoCl_2{\cdot}6H_2O$ 8.16 mg, Na_2SeO_3 4.47 mg.

2. Materials and Methods

2.1. Experimental Feed. The frozen fresh miscellaneous fish used in the experiment was chilled mackerel (*Scomber japonicus*) purchased from the aquatic product market in Zhanjiang City (Guangdong Province, China). The dorsum muscles were cut into pieces and stored at −20°C. According to the feed formula of cobia provided in reference by Weicong et al. [20], the formula feed with a crude protein content of 46% and crude lipid content of 8% was prepared by using a FM, peeled soybean meal as a protein source and fish oil, corn oil, and soybean phospholipid oil as a lipid source (Table 1). The feed raw materials were crushed through a 60-mesh sieve, weighed accurately according to the proportion and mixed. Fish oil and soybean phospholipids were added to the mixture. After the mixture was crushed through a 60-mesh sieve, the oil pellet particles were added with an appropriate amount of water, mixed evenly for the second time, and then processed into a granular feed with a particle size of 1 cm. Then put it in a steam constant temperature room at 80°C for 30 min, dry it in

Table 2: The main nutritional components of formulated diet and chilled chub mackerel (dry matter basis, %).

Nutritional component	Formulated diet	Chilled chub mackerel
Moisture	10.98 ± 0.52	73.56 ± 1.65
Crude protein	46.85 ± 1.25	21.56 ± 1.09
Crude lipid	8.02 ± 0.43	16.46 ± 0.69
Ash	9.85 ± 0.78	1.69 ± 0.38

a cool air-conditioned room, and store it in a sealed bag at −20°C. The main nutritional components of the two experimental feeds are shown in Table 2.

2.2. Experimental Animals and Feeding Management. The cobia juveniles used in the experiment were purchased from an aquaculture company (Hainan Blue Grain Technology Co., Ltd., Sanya, China), the same batch of juveniles artificially cultivated in 2022. The experiment was carried out at the base of Guangdong Evergreen Feed Industry Co., Ltd. The cobia was transported to the breeding base by a special live fish transport vehicle and placed in a 1,000 L culture tank for 2 weeks. A commercial compound feed is fed to juvenile cobia purchased from hatcheries. The juvenile cobias were acclimated to eating chilled fish before beginning the experiment. During acclimatization, chilled chub mackerel was fed at 8:00 and 17:00 every day. After feeding for 24 hr, juvenile cobia (body weight: 37.54 ± 4.57 g) that were healthy, vigorous, and of the same size were randomly selected and divided into two treatment groups (fresh frozen fish group and formulated diet group). There were five replicates in each treatment group, with 30 fish in each replicate. During the experiment, the compound feed and chilled mackerel were fed twice a day (8:00 and 17:00) with apparent satiety, and the feeding continued for about 30 min, and then the residual bait and feces were removed by siphoning. The unconsumed feeds were dried. The breeding experiment period was 12 weeks. The aquaculture facility was an indoor 24 hr continuous aerated water aquaculture system with a water body of 2,000 L. The dissolved oxygen concentration was above 6 mg/L, the natural light cycle; the seawater was sand-filtered sea water, the temperature was 28–30°C, the salinity was 28–30, the ammonia nitrogen was <0.1 mg/L, the nitrite nitrogen was <0.05 mg/L, and the pH was 7.8–8.0.

2.3. Sample Collection and Chemical Analysis. After the completion of the breeding experiment, fasting for 24 hr, six fish were randomly taken from each breeding tank. After being anesthetized with 0.01% MS-222, the body mass and body length were measured and then dissected on the ice, the viscera and liver were taken out, and the mass was recorded. The liver was placed in a 5 mL frozen storage tube and then frozen in liquid nitrogen and then transferred to a refrigerator at −80°C for storage for subsequent determination of liver antioxidant and lipid metabolism indicators.

Liver samples were prepared into a 10% homogenate in an ice-water bath using a homogenizer at the ratio of adding 9 mL of normal saline per gram of tissue to be tested. Then the homogenate was centrifuged at 2,000 r/min at 4°C for 10 min, and the supernatant was divided into 2 mL centrifuge tubes and stored in a 4°C refrigerator for the determination of antioxidant and lipid metabolism indicators.

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), malondialdehyde (MDA), lipoprotein lipase (LPL), fatty acid synthase (FAS), and malate dehydrogenase (MDH) were detected by the kits of Nanjing Jiancheng Bioengineering Institute (http://www.njjcbio.com/). SOD activity/(U/mg prot) was measured by the hydroxylamine method. The activity unit was defined as one activity unit (U) when the SOD inhibition rate of each milligram of tissue protein in 1 mL reaction solution reached 50%. CAT activity/(U/mg prot) is determined by the ammonium molybdate method, and the activity unit is defined as the amount of 1 μmoL H_2O_2 decomposed by 1 mg histone in 1 s as one activity unit (U). The activity of GPX/(U/mg prot) was measured by colorimetry. It was defined as per milligram of protein, deducting the effect of nonenzyme reaction every minute, so the concentration of GSH in the reaction system was reduced by 1 μmol/L as one unit of enzyme activity. The determination of MDA content/(nmol/mg prot) adopts the TBA method and utilizes the condensation reaction of MDA and thiobarbituric acid (TBA). The red product formed has the maximum absorption peak at 532 nm to calculate the content of MDA. Protein content/(g prot/L) was determined by the Coomassie brilliant blue method. The combination of $-NH^{3+}$ on the protein molecule and the anion on the Coomassie brilliant blue dye turned the solution blue. The protein content could be calculated by measuring the absorbance [2–4, 21–23].

LPL, FAS, and MDH were measured by enzyme-linked immunosorbent assay (ELISA). The unit of LPL activity (U/mg prot) was defined as 1 μmol of free fatty acid produced per milligram of tissue protein per hour in the reaction system as one unit of enzyme activity. The FAS activity unit was defined as the oxidation of 1 μmol NADPH per minute per milligram of protein at 37°C as 1 activity unit. The unit of MDH activity (U/mg prot) was defined as one unit of enzyme activity per milligram of protein that catalyzes the transformation of 1 μmol of the substrate into a product within 1 min in the reaction system. The corresponding steps were performed according to the instructions of the kit [2–4, 21–23].

The national standard method was adopted for routine nutrition determination of feed and fish whole body. The crude protein content was determined by the Kjeldahl method, the crude lipid content was detected by the Soxhlet extraction method, the moisture content was determined by drying at 105°C to constant weight, and the ash content was determined by the loss on ignition method at 550°C in a muffle furnace.

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activity, total cholesterol (TC), triglyceride (TG), glucose (GLU) content, calcium concentration, and phosphorus concentration were all measured by the automatic biochemical

analyzer (Hitachi 7020) [24]. Reagents were purchased from Weiteman Biotechnology (Nanjing) Co., Ltd.

2.4. Calculation Formula. Weight gain ratio (WGR %) = $(W_t - W_o)/W_o \times 100$; where W_t = final body weight (%) and W_o = initial body weight (%);

SGR (%/d) = $(\ln W_t - \ln W_o)/t \times 100$; where $\ln W_t$ = final body weight (%) and $\ln W_o$ = initial body weight (%);

Survival rate (SR%) = $N_t/N_o \times 100$; where N_t = number of fish at the end of the test and N_o = number of fish at the beginning of the test;

Condition factor (CF) = $W/L^3 \times 100$; (wet body weight/total body length)

Viscerosomatic index (VSI %) = $Wv/W \times 100$; (wet weight of visceral organ/wet body weight);

Hepatosomatic index (HSI %) = $W_h/W \times 100$; (wet weight of liver/wet body weight);

Protein efficiency ratio (PER %) = $(W_t - W_o)/(W_f \times W_p) \times 100$; wet weight gain (%)/protein ingested (%), where

Feed conversion rate (FCR, g feed/g gain) = dry feed consumed (g)/wet weight gain (g), where the dry matter intake and the total weight of the fish were measured at the end of the experiment;

In the formula, t was the feeding day (d), N_o was the initial tail, N_t was the final tail, W_p was the crude protein content of the feed (%), W_f was the total food intake (g), W_t was the final average weight (g), W_o was the initial average weight (g), Wv was visceral weight (g), W_h was liver weight (g), W was the body weight (g), and L was the body length (cm) [24, 25].

2.5. Data Statistics. The data are expressed as mean ± standard deviation (mean ± SD). The data were plotted using GraphPad Prism 8 software, and the comparison of the two groups of data was performed using the Student's t-test (options: two-tailed, 95% confidence intervals) method for significant difference analysis. $P < 0.05$ means a significant difference and $P < 0.01$ means an extremely significant difference.

3. Results

3.1. Effects of Formulated Diet and Fresh Frozen Fish on the Growth of Cobia Juveniles. The effects of formulated diet and fresh frozen fish on the growth of cobia juveniles are shown in Figure 1. Figure 1 shows that there was no significant difference in the survival rate between the cobia fed with the formulated diet group and the fresh frozen fish group ($P > 0.05$). The results showed that the weight gain rate (WGR), SGR, and FCR of juvenile cobia fed with formulated diet were extremely significantly lower than those of the frozen fresh fish group ($P < 0.01$). SGR, WGR, and FCR decreased by 0.89%/d, 385.7%, and 3.30, respectively, with extremely significant differences between groups (SGR: $t = 8.298$, $df = 8$, $F = 1.130$, $P = 0.0022 < 0.01$; WGR: $t = 7.966$, $df = 8$, $F = 2.855$, $P = 0.0012 < 0.01$; FCR: $t = 5.179$, $df = 8$, $F = 1.100$, $P = 0.0013 < 0.01$). CF decreased by 0.18, and there was a significant difference between the groups ($t = 2.723$, $df = 8$, $F = 3.243$, $P = 0.0261 < 0.05$). VSI, HSI, and PER increased by 1.48%, 0.73%, and 0.41%, respectively, and there were significant differences between the groups (VSI: $t = 3.257$, $df = 8$, $F = 1.517$, $P = 0.0116 < 0.05$, HSI: $t = 2.760$, $df = 8$, $F = 1.307$, $P = 0.0247 < 0.05$, PER: $t = 3.008$, $df = 8$, $F = 1.305$, $P = 0.0169 < 0.05$).

The effect of formulated diet and fresh frozen fish on the nutrient composition of the whole fish of juvenile cobia is shown in Figure 2. It can be seen from Figure 2 that the content of water, crude protein, and crude ash in the cobia-feeding formulated diet group has no significant difference compared with the fresh frozen fish group ($P > 0.05$). The content of crude lipid had decreased by 3.84%, and there was a significant difference between the groups ($t = 2.884$, $df = 8$, $F = 1.570$, $P = 0.0204 < 0.05$).

3.2. Effects of Formulated Diet and Fresh Frozen Fish on Serum Biochemical Indices of Cobia Juveniles. Effects of formulated diet and fresh frozen fish on serum biochemical indices of cobia juveniles were shown in Figure 3. It can be seen from Figure 3 that compared with the fresh frozen fish group, the activity of ALT and the content of Ca of formulated diet group had no significant difference ($P > 0.05$). The activity of AST and glucose content increased by 11.06 U/L and 1.28 mmol/L, respectively, and there were significant differences between the groups(AST: $t = 3.049$, $df = 8$, $F = 3.462$, $P = 0.0158 < 0.05$; GLU: $t = 3.018$, $df = 8$, $F = 1.542$, $P = 0.0166 < 0.05$). Total cholesterol, triglyceride content, alkaline phosphatase activity, and blood phosphorus content decreased by 1.09 mmol/L, 0.72 mmol/L, 11.17 U/L, and 1.03 mmol/L, respectively, with significant differences between the groups (TC: $t = 2.775$, $df = 8$, $F = 1.326$, $P = 0.0241 < 0.05$; TG: $t = 2.836$, $df = 8$, $F = 1.563$, $P = 0.0220 < 0.05$; ALP: $t = 2.737$, $df = 8$, $F = 1.304$, $P = 0.0256 < 0.05$; *P*: $t = 3.599$, $df = 8$, $F = 1.055$, $P = 0.0170 < 0.05$).

3.3. Effects of Formulated Diet and Fresh Frozen Fish on the Liver Antioxidant Ability of Cobia Juveniles. The effects of formulated diet and fresh frozen fish on the antioxidant capacity of cobia juveniles are shown in Figure 4. It can be seen from Figure 4 that there was no significant difference in the catalase activity between the cobia fed with formulated diet and fresh frozen fish ($P > 0.05$). Compared with the frozen fresh fish group, the activities of SOD, GPX, and LPL in the liver of the formulated diet group were significantly decreased ($P < 0.05$) in the fish fed with the formulated feed. The content of MDA and the activity of FAS in the liver were significantly increased ($P < 0.05$) in the group fed with the frozen fresh fish. SOD activity decreased by 8.43 U/mg prot, and there was a significant difference between the groups($t = 2.599$, $df = 8$, $F = 1.540$, $P = 0.0317 < 0.05$). GPX activity decreased by 47.52 U/mg prot, and there was a significant difference between the groups($t = 3.987$, $df = 8$, $F = 3.384$, $P = 0.0040 < 0.01$). The content of MDA increased by 1.07 nmol/mL, with a significant difference between groups($t = 2.712$, $df = 8$, $F = 3.075$, $P = 0.0266 < 0.05$).

3.4. Effects of Formulated Diet and Fresh Frozen Fish on Lipid Metabolism of Cobia Juveniles. The effects of formulated diet and fresh frozen fish on the lipid metabolism of cobia

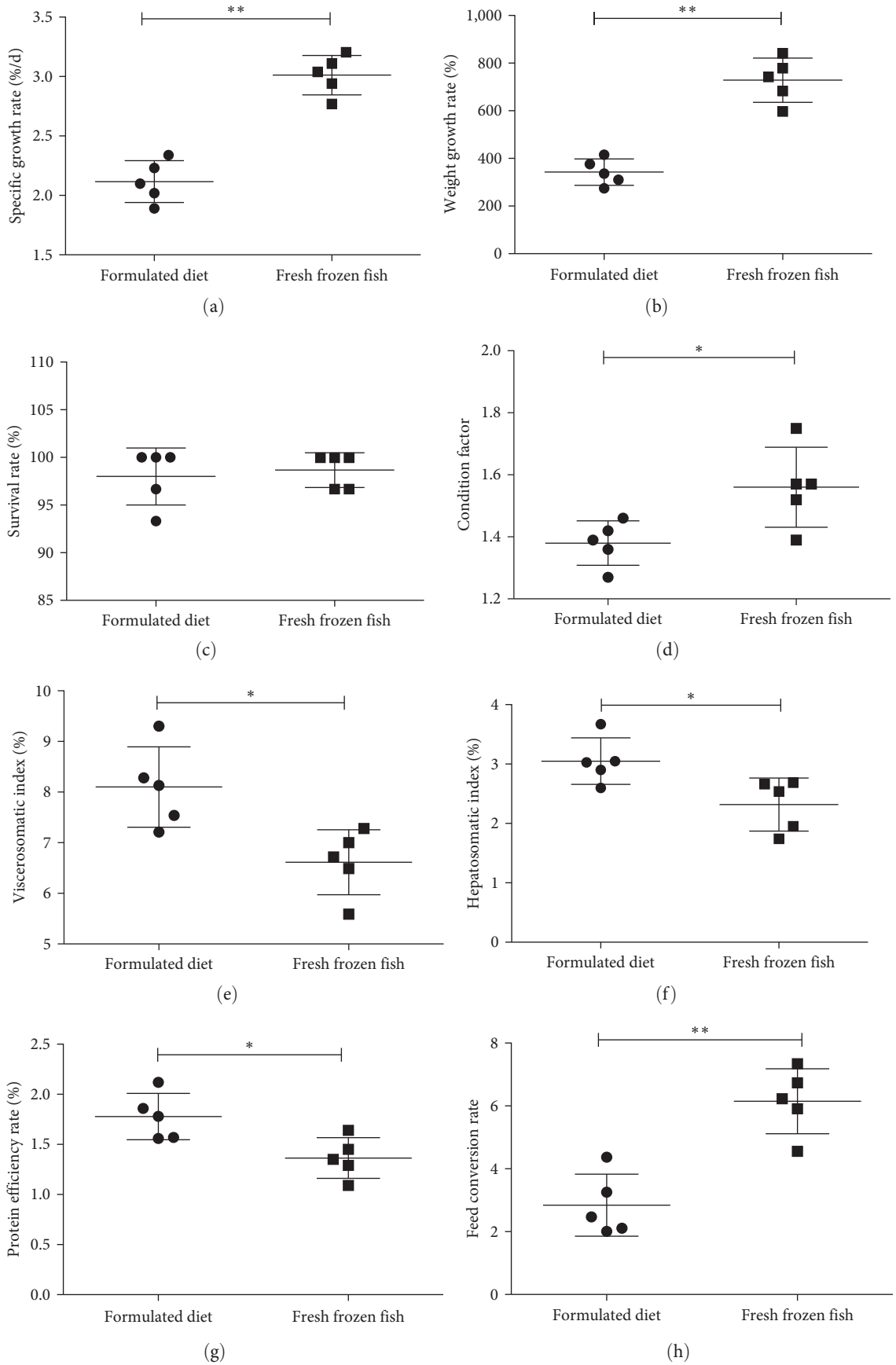


Figure 1: Effects of formulated diet and fresh frozen fish on growth of cobia juveniles (a–h). Notes: * shows significant difference and ** shows extremely significant difference.

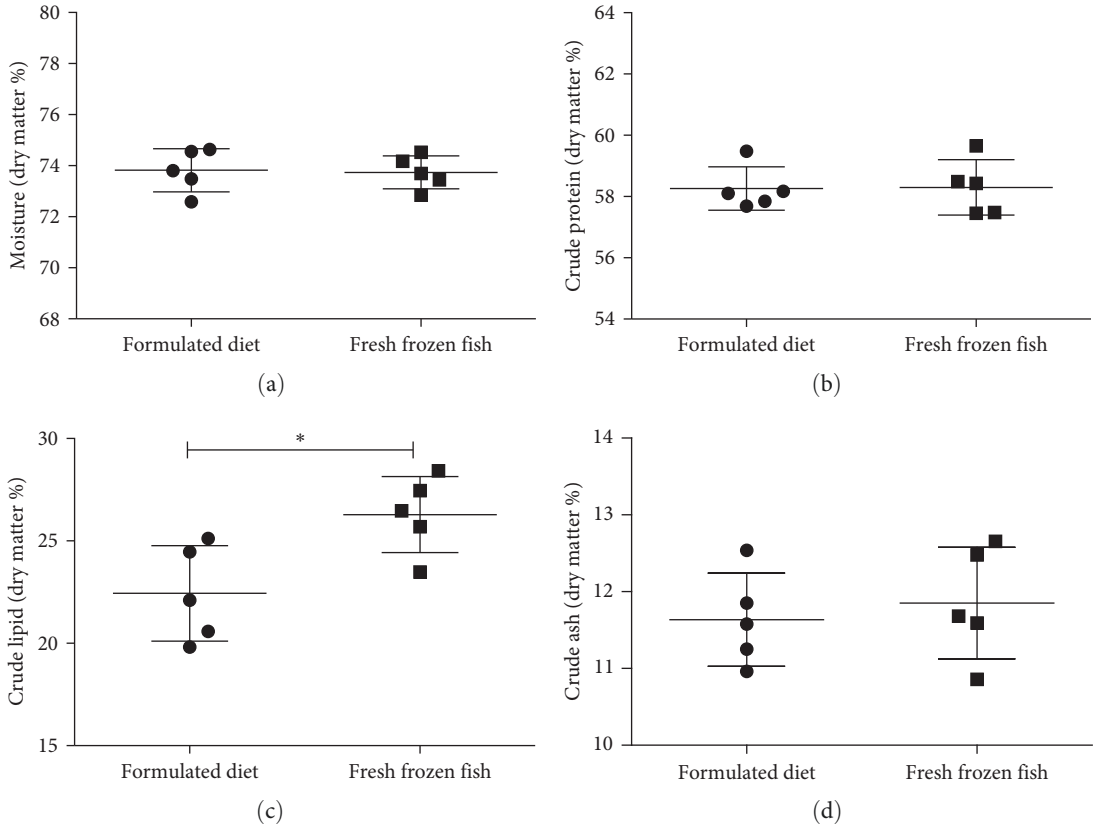


Figure 2: Effects of formulated diet and fresh frozen fish on whole body components of cobia juveniles (a–d). Notes: * shows significant difference and ** shows extremely significant difference.

juveniles are shown in Figure 5. It can be seen from Figure 5 that there was no significant difference in MDH activity between the cobia fed with formulated diet and fresh frozen fish ($P > 0.05$). LPL activity decreased by 231.3 U/mg prot, and there was a very significant difference between the groups($t = 4.461$, $df = 8$, $F = 1.303$, $P = 0.0021 < 0.01$). FAS activity increased by 223.1 U/mg prot, and there was a significant difference between the groups($t = 3.923$, $df = 8$, $F = 1.248$, $P = 0.0175 < 0.05$).

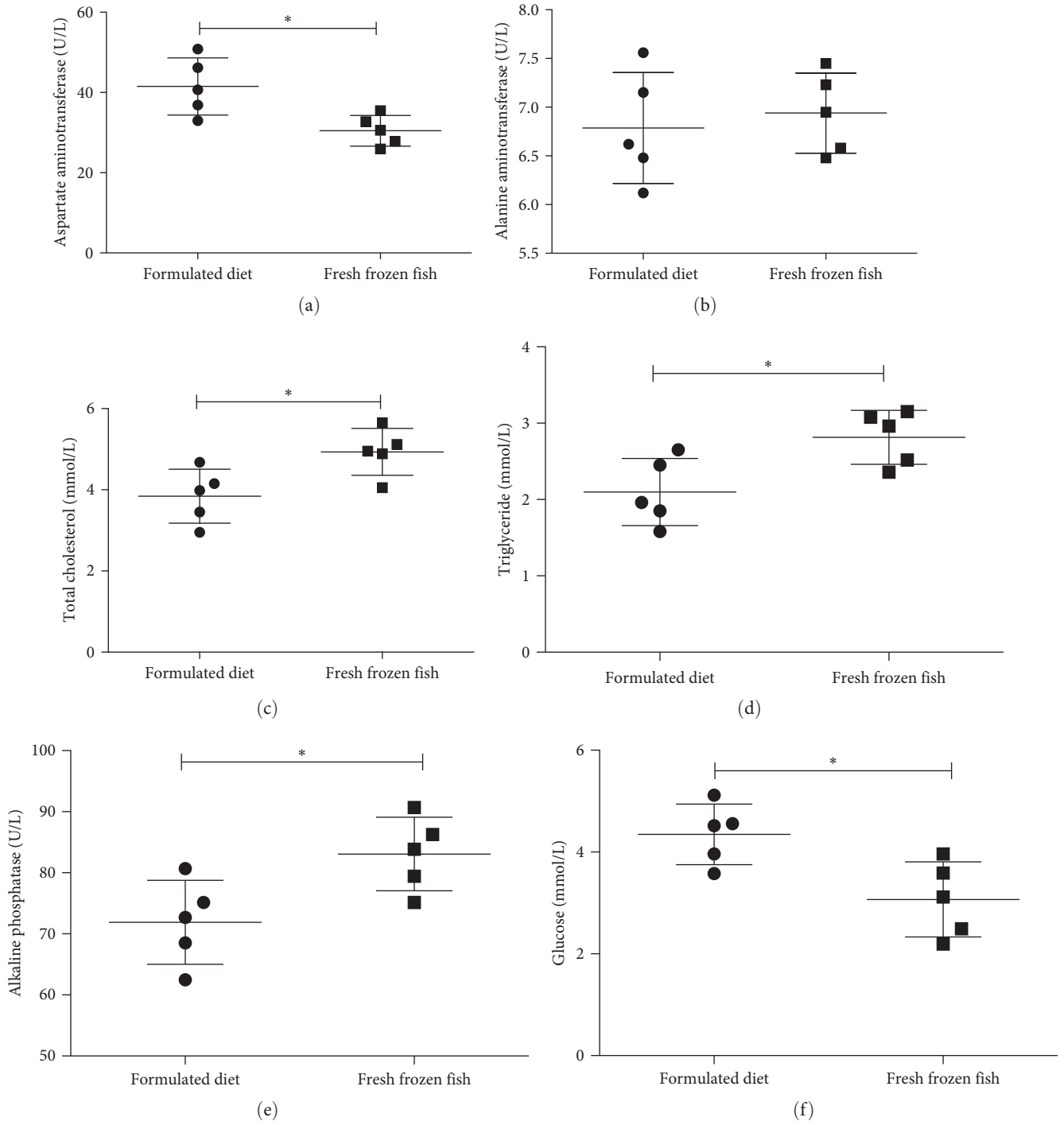
4. Discussion

4.1. Effects of Formulated Diet and Fresh Frozen Fish on the Growth of Cobia Juveniles. Feed is the main material and energy source for the cultured fish. Its nutrition and quality safety are conducive to maintaining fish growth and enhancing disease resistance and disease prevention ability. Therefore, the feed quality evaluation should assess the growth rate, aquaculture yield, feed utilization efficiency, and the impact on cultured fish's health, metabolism, and physique [26].

The experiment showed that the two kinds of feed (formulated diet and frozen fresh fish) had no significant effect on the survival rate of juvenile cobia. In contrast, the SGR and WGR of juvenile cobia in the formulated diet group were 0.89%/d and 385.7% lower than those in the fresh frozen fish group, respectively, indicating that the formulated diet was not conducive to improving the growth of cobia. This was consistent with the juvenile of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [27], turbot (*Scophthalmus maximus*) [28], and largemouth bass (*Micopterus salmoides*) [29]. These results showed that the utilization effect of carnivorous cultured fish on fresh frozen fish was better than that of formula feed, indicating that the nutritional characteristics of formula feed could not adapt to the rapid growth of carnivorous fish. In this experiment, it may be that the frozen fresh fish had good palatability and a strong food-attraction effect, which was more conducive to the digestion and absorption of juvenile cobia. However, the FCR of the frozen fresh fish group was 6.14, which was significantly higher than the 2.84 of formulated diet group. This was due to the high water content of frozen fresh fish and the low-conversion rate. Under the condition of meeting their own nutritional needs, the cobia will eat more quality frozen fresh fish to meet their growth and development.

The protein efficiency rate (PER) is a measure of how efficiently an organism utilizes dietary protein for growth [25]. It is calculated by dividing the weight gain by the amount of protein consumed. A higher PER indicates better protein utilization efficiency [24, 25]. In the context of this study, the PER results indicates how well the diets used meet the protein requirements of the juvenile cobia. The higher PER in the group fed the formulated diet suggest that the diet provides sufficient and digestible protein for growth and that the formulated diet is better suited for the cobia protein needs.

HSI is a measure of the liver's relative size compared to the whole body. Changes in HSI can reflect alterations in



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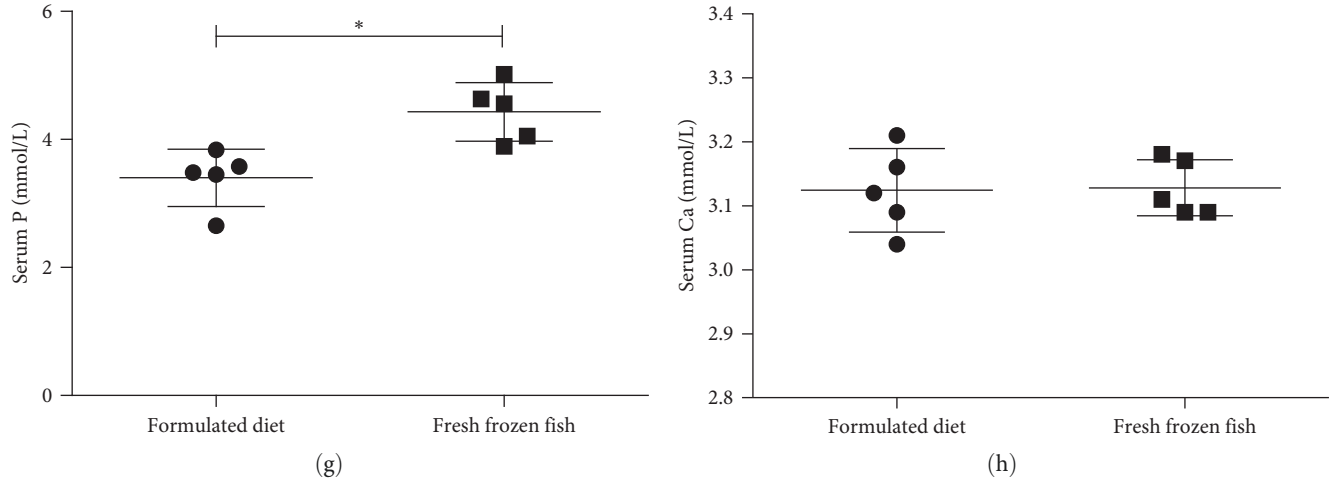

FIGURE 3: Effects of formulated diet and fresh frozen fish on serum biochemical indices of cobia juveniles (a–h). Notes: * shows significant difference and ** shows extremely significant difference.

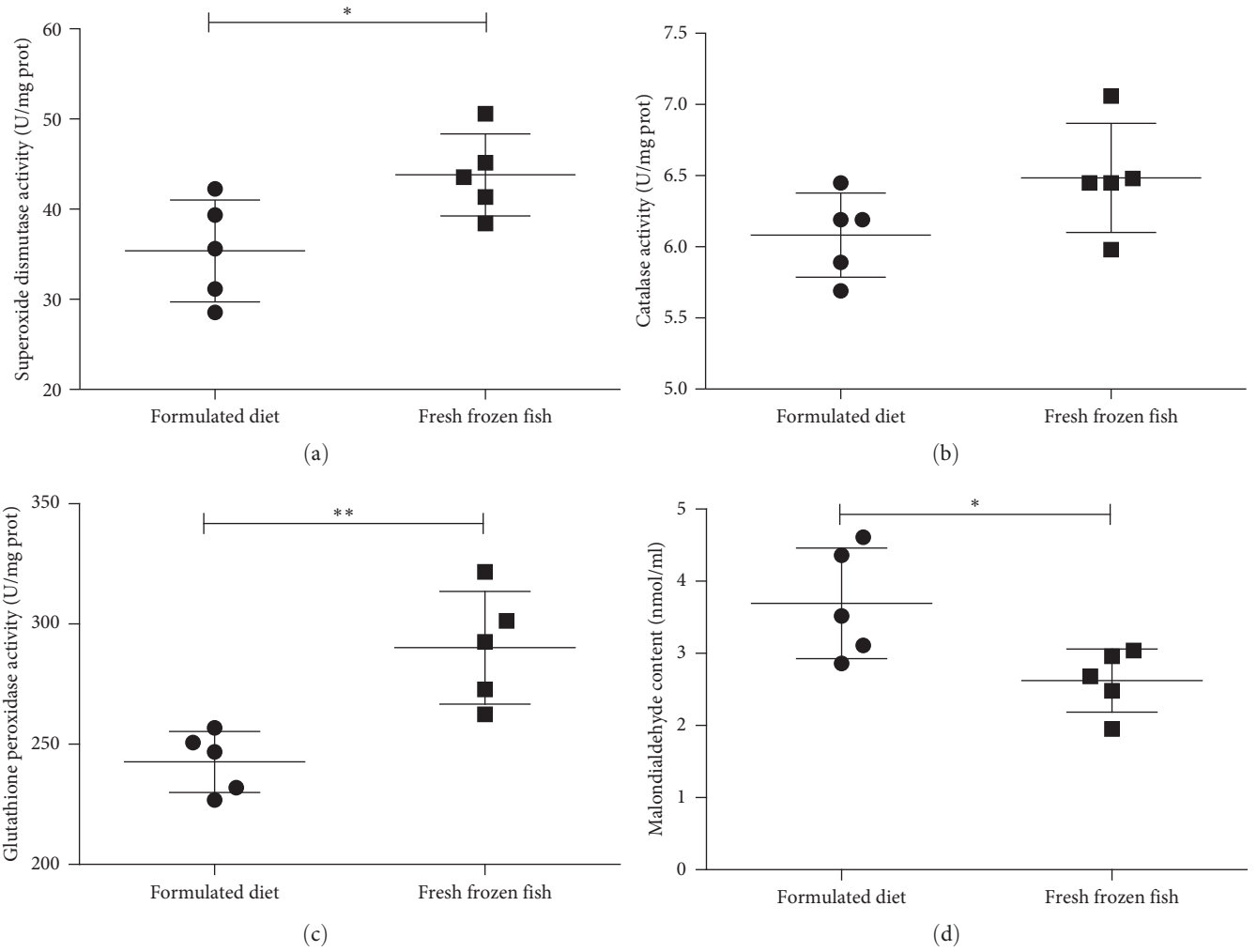

FIGURE 4: Effects of formulated diet and fresh frozen fish on liver antioxidant ability of cobia juveniles (a–d). Notes: * shows significant difference and ** shows extremely significant difference.

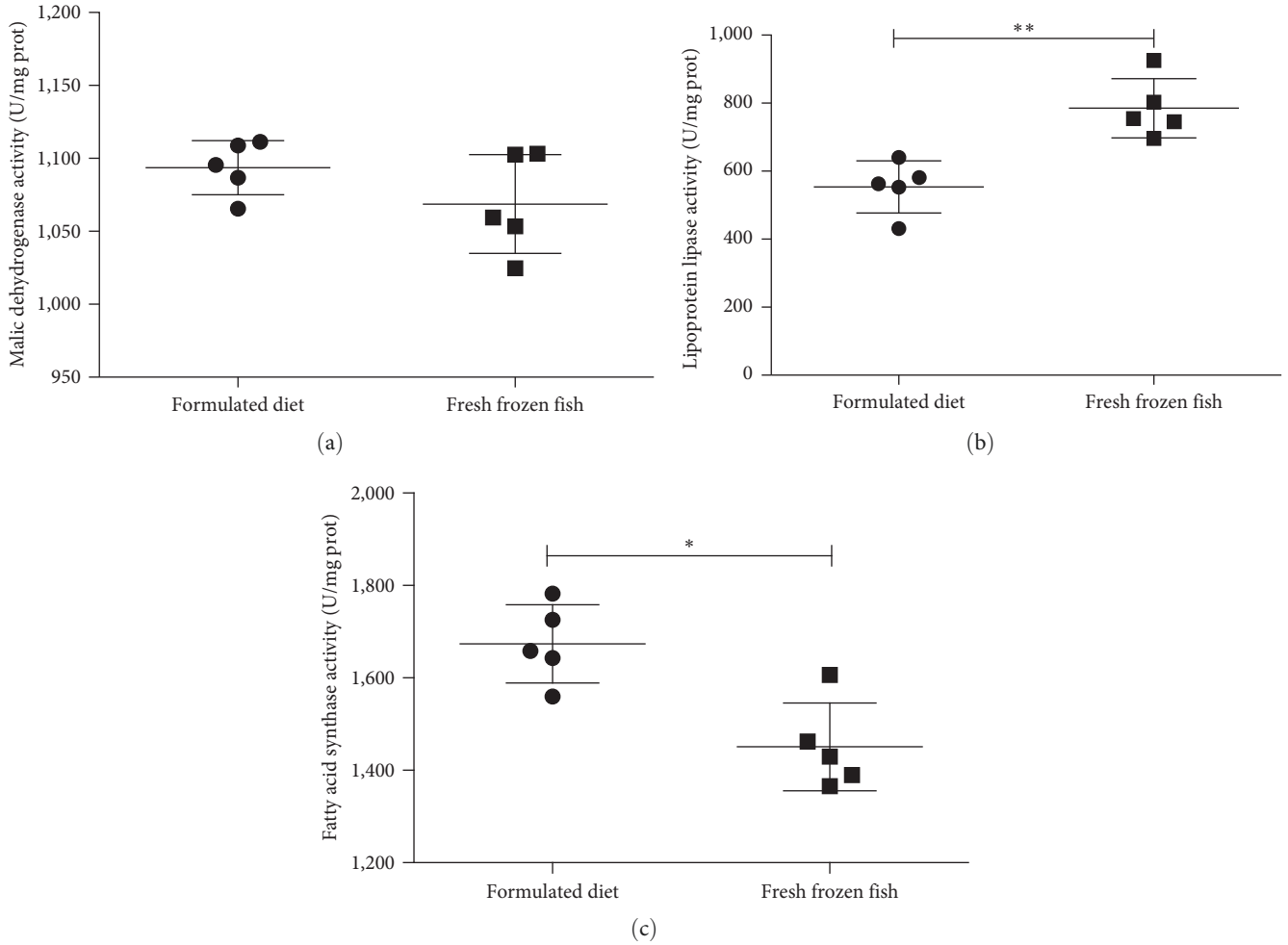

Figure 5: Effects of formulated diet and fresh frozen fish on lipid metabolism of cobia juveniles (a–c). Notes: * shows significant difference and ** shows extremely significant difference.

liver health, metabolic activity, or energy allocation [30, 31]. In the present study, changes in the HSI indicate how the different diets affect the liver health of the cobia. An increase in HSI might suggest liver enlargement, which could be due to increased energy storage, detoxification, or other metabolic processes as the results shows in the present study when the fish were fed with the formulated diet [30, 31]. Conversely, a decrease in HSI indicates that the liver is under less stress and the fish are allocating energy more toward growth, hence the frozen fresh fish is better suited for the cobia HSI needs.

VSI is a measure of the visceral organs' (such as the heart, stomach, and intestines) relative size compared to the whole body [32]. Changes in VSI can indicate shifts in energy allocation, digestion efficiency, or physiological stress [32–34]. In the present study's context, VSI changes provide insights into how the different diets affect the fish's digestion, energy allocation, and overall health. An increase in VSI may suggest that formulated diet is causing the fish to allocate more energy toward digestion, possibly due to differences in nutrient composition or digestibility. A decrease in VSI indicates more efficient digestion and nutrient absorption and that the frozen fresh fish is better suited for the cobia VSI needs.

Currently, in the production of cobia cage aquaculture in China, frozen fresh miscellaneous fish was mainly fed, which restricted the sustainable development of the cobia aquaculture industry. On the one hand, due to the scarcity of fishery resources, there were fewer and fewer bait fish in the ocean, which made it more difficult to catch, and the cost rose accordingly. Moreover, the large number of small miscellaneous fish will also cause a certain degree of damage to the natural ecology of the ocean. The study has shown that feeding chilled miscellaneous fish will also increase the pollution of aquaculture water [35]. On the other hand, chilled miscellaneous fish carries certain pathogens and induces diseases, and high-temperature seasons can easily lead to fatty acid spoilage. Stale miscellaneous fish would also cause intestinal diseases of farmed fish and aggravate the occurrence of diseases. Therefore, the feed preparation technology of cobia should be adjusted and optimized in combination with the nutritional composition characteristics of ice fish, which could reduce the dependence of cobia farming on ice fish,

which was of great significance to promoting the healthy development of seawater aquaculture.

4.2. Effects of Formulated Diet and Fresh Frozen Fish on Serum Biochemical Indices of Cobia Juveniles. The physiological status of the body and nutritional status are reflected in serum biochemical indicators [19]. CF was an index reflecting the degree of fish fatness. In this experiment, the CF of the frozen fresh fish group was significantly higher than that of the formulated diet group, indicating that the body shape of the cobia in the frozen fresh fish group was plumper. In contrast, the body shape of the formulated diet group was slender, which was similar to that of hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [27], turbot (*Scophthalmus maximus*) [28], and largemouth bass (*Micropterus salmoides*) [29] found the same results. Previous studies have also confirmed that European sea bass (*Dicentrarchus labrax*) [36], giant croaker (*Nibea japonica*) [37], and golden pompano (*Trachinotus ovatus*) [38] ingesting high-sugar diet was easy to cause protein digestion or glucose metabolism disorders, which would cause fat deposition in the liver, increase the ratio of liver to the body, and cause damage to the liver.

Fish blood physiological and biochemical indicators were closely related to feed nutrition levels; therefore, changes in blood physiological and biochemical indicators can reflect the body's metabolism, nutrition, and physiological status [18]. As a result of various factors causing liver damage, ALT and AST are infiltrated into the bloodstream, increasing both enzymes' concentration in the serum. Therefore, increased serum ALT and AST activity indicates some degree of impairment of liver function. Free radicals are produced during lipid metabolism by oxygen-demanding organisms, which are a major contributor to the oxidative damage and antioxidant imbalances [39]. Under normal conditions, the activity of ALT and AST in the body's blood was low. Only when the tissue cells were destroyed and damaged would the activity of transaminase in the blood be enhanced. This experiment found that the activity of AST in the serum of juvenile cobia was increased after being fed with compound feed, which indicated that feeding compound feed had certain damage to the liver of cobia, which reflected the poor physiological adaptability of cobia to compound feed.

The contents of TG and CHO in serum can also reflect the metabolism of fish lipids. Studies have shown that after the liver of fish is damaged, TG and CHO cannot be transported out in time and accumulate in the liver, which reduces the TG and CHO in the blood because when the lipid in the liver cells cannot be transported out with the blood in time, it would cause lipid accumulation in the liver. Therefore, when the lipid content in the liver increases, the lipid content in the blood would decrease [27]. In this experiment, the content of TG and CHO in the formula feed group was significantly lower than that in the ice-fresh fish group. It may be that the liver was damaged to a certain extent and further affected the transport of TG and CHO. Similar results were found in the hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [27], *Scophthalmus maximus* [28], *Micropterus salmoides* [29], and GIFT *Oreochromis niloticus* [40]. Based on the analysis of serum biochemical indexes and morphological indexes, feeding formula feed has a certain impact on the liver of young cobia fish. The reasons for analysis were as follows: (1) the breed of cultured fish was different, and cobia belonged to carnivorous fish. Its adaptability to natural food was stronger than that of artificial formula feed. (2) The high content of starch in formula feed may affect the lipid metabolism of cobia. Previous studies have shown that the utilization rate of carbohydrates in carnivorous fish is very low. Wang et al. [15] pointed out that with the decrease in feed carbohydrate level, the cobia's VSI, HIS, and liver lipid content showed a downward trend. The iced fresh fish used in this experiment had higher fat content. However, the VSI, HIS, and AST activities were significantly lower than those in the compound feed, indicating that cobia juveniles could tolerate high-lipid feed. High-lipid content was not the main reason for the tendency of cobia fatty liver in this experiment. Studies have shown that seawater carnivorous fish have poor tolerance to high glucose, which was one of the reasons leading to the tendency of fatty liver [27]. Therefore, attention should be paid to the addition of sugar energy feed in the production of cobia. (3) There was a lack of antifatty liver factors in formula feed. The amino acid composition of frozen fresh fish was balanced, and the fatty acids were mostly unsaturated fatty acids. Iced fresh fish contains highly unsaturated fatty acids, methionine, lysine, trace elements, and other antifatty liver factors to promote fat metabolism [41]. Therefore, attention should be paid to the appropriate addition of relevant anti-adipogenic factors in the formula feed for juvenile cobia.

ALP is a multifunctional enzyme that directly participates in the body's transfer and metabolism of phosphate groups. Its activity is an indicator of the intensity of phosphorus metabolism. ALP is also an important component of lysosomal enzymes and plays an important role in the immunity of fish [42]. Studies have shown that phosphorus deficiency in fish can lead to decreased ALP activity [43]. This experiment found that after feeding cobia with frozen fresh fish, the phosphorus content in serum increased significantly, which in turn led to an increase in plasma ALP activity, indicating that feeding iced fish can improve the immune ability of cobia, which may be the reason for the increase in ALP activity. The increase of serum phosphorus content strengthens the phosphorylation in the process of fish material metabolism. It weakens gluconeogenesis, which is one of the reasons why the cobia grows faster than the compound feed after eating iced fish.

4.3. Effects of Formulated Diet and Fresh Frozen Fish on Liver Antioxidant Capacity and Lipid Metabolism of Cobia Juveniles. An antioxidant system in the body is responsible for scavenging free radicals [44]. The main antioxidant indicators of fish include SOD, CAT, GSH-Px, MDA etc., and their main function is to scavenge superoxide free radicals, including superoxide anion (O_2^-), and hydrogen peroxide [45]. GSH-Px and SOD are antioxidant enzyme systems that scavenge free radicals and prevent oxidative damage to cellular macromolecules and organelles [46]. The concentration

of MDA in biological membranes reflects the degree of cellular damage induced by lipid peroxidation caused by free radicals [47]. SOD plays a vital role in the balance of oxidation and oxidation resistance of the body. It can act on superoxide anion free radicals, convert the superoxide anion free radicals into hydrogen peroxide in the body, and protect cells from damage; CAT can specifically remove excess hydrogen peroxide in the body and protect cells from oxidative damage [48]. As the main metabolite of lipid peroxidation reaction, MDA is an important indicator of oxidative stress. Its content can not only indirectly reflect the content of reactive oxygen free radicals but also reflect the intensity of lipid peroxidation reaction in tissue cells and the increase and decrease of lipid peroxide [49]. Under pathological or stress conditions, fish can produce a large number of free radicals, which would undergo a lipid peroxidation reaction with unsaturated fatty acids to form lipid peroxidation products such as MDA, which would have a toxic effect on fish [50]. In this study, juvenile cobia fed with compound feed significantly increased liver MDA content, indicating that lipid peroxidation occurred in the liver. This is consistent with the findings on largemouth bass (*Micropterus salmoides*) [29] and Nile tilapia (*O. niloticus*) [51]. In addition, the activities of serum SOD and GSHPx decreased significantly after the cobia fed the compound feed in the experiment, which indicated that while the compound feed increased the MDA content of the fish, the antioxidant defense system in the fish was also damaged, which in turn weakened the body's ability to scavenge free radicals. The increase in serum AST activity further confirmed that the compound feed caused obvious damage to the liver of cobia.

In aquatic animals, lipids play a vital role in their growth. In addition to providing energy for growth, it also facilitates the absorption of fat-soluble nutrients [52–54]. By using lipids in aquaculture, feed protein is saved and nitrogen is reduced [55]. Protein in the body acts as an energy source when fat in the feed isn't sufficient to meet the feed's energy requirements for aquatic animal growth, thereby reducing anabolism [52]. Growth and development will be negatively affected by a deficiency in essential fatty acids. Also, this condition causes aquatic animals to lack essential fatty acids, which can affect their growth and even cause diseases of the digestive system [56]. The excessive lipid content in feed, however, causes aquatic animals to consume excessive amounts of fat and to accumulate fat in their muscles and liver, leading to liver damage and metabolic problems [52, 57]. Consequently, the body's antioxidant capacity and aquatic product quality are reduced. Thus, it is imperative to develop feed additives to relieve fatty liver disease [52]. As lipid metabolism is not fully functional in marine larvae, they require lipids from their diets to grow and develop [58–61]. Hence, a thorough understanding of how lipids are transported and accumulated in fish is necessary [58].

The liver is the most important organ of fish lipid metabolism and plays an important regulatory role in the process of fish lipid metabolism [62]. Lipid synthesis and decomposition are two basic processes of fat metabolism. Fatty acids in the feed are absorbed and transported to storage sites [62]. During the entire storage process, fatty acids are generally in the form of triglyceride-binding proteins to form chylomicrons, which are catalyzed by various lipases such as LPL, FAS, MDH, and glucokinase [62]. LPL mainly plays a role in the lipolysis stage. It decomposes triglycerides from feed to produce glycerol and free fatty acids and acts as a rate-limiting enzyme in this process. In this experiment, feeding iced fresh fish significantly increased the liver LPL activity of cobia, which may be due to the fact that chub mackerel with high lipid provided a large amount of lipid for cobia, accelerated the metabolism of the body, required a large amount of LPL to decompose triglycerides, thus promoting the liver to secrete more LPL, leading to a significant increase in liver LPL activity of cobia fed iced fresh fish compared with that fed formula feed. This is similar to the hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [27] and turbot (*Scophthalmus maximus*) [28]. FAS is the key enzyme of endogenous fatty acid synthesis, and its activity is of great significance for controlling animal body fat deposition [63]. Feed nutrients have a significant impact on the activity of fish FAS. Han et al. [64] studied that feed with high-fat levels inhibits the expression of the FAS gene and reduces its activity. The results of this study also showed that feeding frozen fresh fish significantly reduced the activity of FAS in the liver of cobia, which may be due to the high-fat content of the iced fresh chub mackerel. The cobia does not need to synthesize a large number of fatty acids to participate in the synthesis and deposition of fat, thus leading to a significant decrease in the activity of FAS in the liver. The activity of MDH was directly related to the production of reduced nicotinamide adenine dinucleotide phosphate and then affects the synthesis of body fat. In this experiment, although the MDH activity of the liver of the cobia was decreased in the group fed with iced fresh chub mackerel, there was no significant difference between the group fed with formula diet, which was similar to that of the hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) [27]. The research results are consistent with those of *Scophthalmus maximus* [28], which may be due to different fish species and different adaptions to formula feed.

This research aims to contribute to the existing knowledge on cobia nutrition and feeding management, providing valuable insights for fish farmers, aquaculture researchers, and nutritionists in the field. The findings from this study will help in developing tailored feeding strategies that promote optimal growth, health, and nutrition of juvenile cobia, ensuring the sustainability and profitability of cobia aquaculture industry in the future. The findings also provide a reference for the healthy and efficient breeding of cobia and the development of compound feed.

5. Conclusion

This study investigated the effects of formulated diet and frozen fresh fish on the growth, serum biochemical indexes, liver antioxidant, and lipid metabolism of juvenile cobia (*Rachycentron canadum*). The results showed that the WGR, SGR, and FCR of juvenile cobia fish fed with formulated diet

were extremely significantly lower than those of the frozen fresh fish group ($P<0.01$). Additionally, the PER, HIS, and VSI were significantly higher than those of the frozen fresh fish group ($P<0.05$). The serum aspartate aminotransferase activity and sugar content of cobia in the formulated diet group were significantly higher than those in the frozen fresh fish group ($P<0.05$). In contrast, the total cholesterol, triglyceride content, alkaline phosphatase activity, and phosphorus content were significantly lower than those in the frozen fresh fish group ($P<0.05$).

Furthermore, the study found that the liver antioxidant and lipid metabolism of the cobia were significantly affected by the diet. Compared with the frozen fresh fish group, the activities of SOD, GPX, and LPL in the liver of the formulated diet group were significantly decreased ($P<0.05$), indicating better antioxidant status with the frozen fresh fish. The content of MDA and the activity of FAS in the liver were significantly increased ($P<0.05$). Additionally, the group fed with the formulated diet had lower levels of liver triglyceride (TG) and TC compared to the group fed with frozen fresh fish, suggesting better lipid metabolism.

Overall, the study highlights the importance of using frozen fresh fish for the growth and health of juvenile cobia, as well as the potential benefits of frozen fresh fish on the antioxidant and lipid metabolism of fish. This information is valuable to the aquaculture industry as it can help optimize the growth and health of cobia, a commercially important fish species. Additionally, the findings of the study can contribute to the development of new and improved frozen fresh fish for other fish species, potentially leading to more sustainable and efficient aquaculture practices. Therefore, this study's findings are significant and relevant to both the scientific community and the aquaculture industry, making it essential for advancing our understanding of the nutritional requirements and growth performance of juvenile cobia.

Data Availability

Data supporting the results of this study will be provided upon reasonable request.

Ethical Approval

All animals used in this project were handled following the Animal Welfare Act, the PHS Animal Welfare Policy, the National Institutes of Health's Guide for Care and Use of Laboratory Animals, as well as the policies and procedures of the People's Republic of China, Guangdong Province, and Guangdong Ocean University. The study was conducted under the rules and regulations of Guangdong Province, China, and the guidelines for the care and use of laboratory animals at Guangdong Ocean University (approval number: GDOU-LAE-2022-035).

Conflicts of Interest

Rui-tao Xie worked for Guangdong Evergreen Feed Industry Co. Ltd. in Zhanjiang, 524003, China. There are no commercial or financial relationships between the remaining authors that could be construed as a potential conflicts of interest.

Authors' Contributions

Jian-sheng Huang was responsible for the project administration, data collection, formal analysis, processing, and editing. Dian-yu Chen and Jing-hui Jin were involved in the data curation. Rui-Tao Xie provided experimental guidance. Yi Lu and Eric Amenyogbe revised the experimental design, supervised the experiment, and provided funding. Jian-sheng Huang, Yi Lu, and Eric Amenyogbe were responsible for writing of the original draft and reviewing.

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