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Research Article

Evaluation of Optimal Dietary Protein Levels for Juvenile Hybrid Abalone under Three Temperatures: Growth Performance, Body Composition, Biochemical Responses, and Antioxidant Capacity

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We studied the effects of dietary protein levels and water temperatures on growth performance, body composition, serum biochemistry, and digestive gland antioxidant enzyme activities of juvenile hybrid abalones *Haliotis discus hannai*♀ × *H. fulgens*♂ (1.47 ± 0.03 g; 20.73 ± 0.16 mm). A 3 × 6 factorial design feeding trial was conducted with three water temperatures (19°C, 23°C, and 27°C) and six protein levels (152.5, 202.5, 252.6, 302.6, 352.7, and 402.7 g/kg) for 90 days. Dietary protein levels and temperature significantly affected the growth performance of the hybrid abalones, but there was no interaction effect except for daily increment in shell length. Body moisture decreased with increasing water temperature, and crude protein showed a contrary tendency. Crude lipid and ash were not affected by dietary protein level or water temperature. The alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities and the triglyceride content in serum increased with increasing water temperature, whereas the opposite was true for albumin activity and total protein and glucose contents. Total cholesterol contents decreased with increasing dietary protein levels. Dietary protein levels and water temperature did not affect contents of high-density lipoprotein cholesterol and low-density lipoprotein cholesterol ($P > 0.05$). A significant interaction was observed between dietary protein levels and water temperature in all antioxidation parameters (reactive oxygen species, total antioxidant capacity, protein carbonyl, superoxide dismutase, catalase, glutathione s-transferase, and glutathione peroxidase except for malondialdehyde content). Based on specific growth rate, the optimal dietary protein levels for juvenile hybrid abalones were 333.1, 318.6, and 306.3 g protein/kg diet at 19°C, 23°C, and 27°C, respectively. These findings will help to develop multidiet feeding strategies at different water temperatures throughout the culture period of juvenile hybrid abalones.

1. Introduction

The total production of abalone in China 2020 was 203,485 tons. This was 90% of the farmed abalone production in the world, and its total value was more than 3.2 billion USD [1, 2]. Pacific abalone (*Haliotis discus hannai*) was the main cultured abalone species in China over the previous two decades [3, 4]. However, slow growth and high mortality in summer are serious issues for abalone culture [5, 6]. In 2015, hybridization between Pacific abalone *H. discus hannai* and green abalone *H. fulgens* was achieved, and the hybrid abalone *H. discus hannai*♀ × *H. fulgens*♂ (herein referred to as DF) showed positive heterosis in growth and high temperature resistance compared to the parental species. These improvements make it a superior variety for commercial culture in China [7]. Although hybridization improved the abalone growth rate, optimal production also depended on there being appropriate physicochemical properties in the culture environment and optimum nutrition [8, 9].

In nutrition studies of aquatic animals, determining the optimal dietary protein level for growth is important because protein is the most expensive and principal nutrient in aquatic animal diets. Protein levels substantially affect animal growth and feed cost [10–12]. Many studies have evaluated the optimum dietary protein level for abalone, including *H. discus hannai* [13, 14], South African abalone (*H. midae*) [15–17], greenlip abalone (*H. laevigata*) [18, 19], blacklip abalone (*H. rubra*) [20], *H. tuberculata* [14], ear abalone (*H. asinina*) [21], black-footed abalone (*H. iris*) [22], and pink abalone (*H. corrugata*) [23]. The optimal protein levels for abalone varied among different species and ranged from 27% to 47%. In 2018, the hybrid abalone *H. discus hannai*♀ × *H. fulgens*♂ was granted "new variety" certification by the Chinese Ministry of Agriculture due to its fast growth rate and increased resistance to high temperatures, and it has been cultured on a large-scale from 2019 to 2021. However, there is no information on the optimal protein level for the hybrid abalone. Insufficient dietary protein in feed adversely affects the growth of aquatic animals. Excess protein in feed wastes feed, increases nitrogen waste output, and may also impair animal growth [24–27]. The growth of aquatic animals depends on the metabolism and physiological status as well as oxidative stress status, which could be reflected by serum biochemistry and antioxidation parameters, respectively [28, 29]. It has been reported that dietary protein level could regulate the serum biochemical indicators and antioxidative capacity in aquatic animals [30]. Moreover, we found a 56.4% increase of body weight in hybrid abalone compared to the Pacific abalone under the same culture conditions. However, the protein level of the commercial feed used for the hybrid abalone was referred to that used for the Pacific abalone, which is unreasonable. Therefore, it is important to determine the optimal dietary protein level for the hybrid abalone.

The optimal protein level of aquatic animals is dependent on rearing conditions such as salinity, dissolved oxygen, and water temperature. Among these, water temperature is the most important abiotic factor for ectotherms such as abalone [31–33]. For example, as water temperature increased from 14°C to 18°C and 22°C, the optimal dietary protein level for *H. laevigata* increased from 29% to 32% and 34%, respectively [19]. The optimal protein level for *H. asinina* was higher when the water temperature was low [21]. To our knowledge, previous studies also demonstrated that water temperature, as a stressor, had significant effects on body composition, serum biochemistry indices, and antioxidant capacity of aquatic species [10, 34]. No information is currently available on the effects of water temperature on the optimal protein level for hybrid abalone. This information is necessary to determine the interaction of water temperature and dietary protein level on hybrid abalone.

The objective of this study was to investigate the interactive effects of dietary protein levels and water temperatures on growth performance, body composition, serum biochemistry, and digestive gland antioxidant enzyme activities of hybrid abalone. The results will help to modulate feed formulation for hybrid abalone culture at different water temperatures and use multidiet feeding strategies throughout the culture period. Besides, this study will, to some extent, provide baseline nutrition data for the references to research and development of the hybrid abalone feed formulation.

2. Materials and Methods

All animal care and handling procedures in present study were approved by the Animal Care Committee of Xiamen University.

2.1. Experimental Diets. Six isoenergetic (16.52 kJ/g) and isolipidic (39.52 g/kg) diets with graded protein levels were formulated, and the actual protein levels of six experimental diets were 152.5, 202.5, 252.6, 302.6, 352.7, and 402.7 g/kg. The ingredients and approximate compositions of these diets are shown in Table 1. All experimental diets were processed by Fuzhou Promarine Biotechnology Co., Ltd., Fujian Province. Prior to the preparation of the experimental diets, all ingredients were crushed and passed through a 180 micrometre mesh and mixed well according to the ratio of each raw material. All of the ingredients were mixed thoroughly with 20% water to create a dough. The doughs were pressed into flakes (length × width × thickness = 4 cm × 3 cm × 1 cm) by a tablet compressing machine. After that, the flakes were steamed for 10 min at 105°C and dried by oven at 60°C for about 8 h until the moisture content in diet was less than 8%. All the diets were stored at −20°C until use.

2.2. Leaching Test. Preweighed experimental diets were placed into 100 μm-mesh screen and placed into cages of three seawater temperature controlling systems, respectively. At the end of the different immersion time intervals (12 h and 24 h), the remaining diets were removed from the cages and dried to constant weight. The leaching loss rate was computed as

$$\text{The leaching loss rate}\,(\%) = \frac{[(L_0 - L_t)]}{L_0} \times 100, \qquad (1)$$

where L_0 is the initial weight of feed and L_t is the final weight (12 h or 24 h) of the feed.

2.3. Feeding Trial. The feeding trial was conducted from May 2021 to September 2021 in a seawater temperature controlled system with sand-filtering seawater at the Fuda abalone farm (Jinjiang, China). The 6-month old juvenile DF was spawned from the same broodstock line and obtained from Fuda Aquaculture Co., Ltd., Jinjiang City, Fujian Province, China. Before the experiments began, the abalone was distributed into three separate seawater temperature controlling systems (8 m × 2.2 m × 0.8 m rectangular concrete ponds and water depth was 0.4 m) and fed a commercial diet (protein, 28%, lipid, 1%) once daily for 14 d, during which the water temperature was raised or lowered slowly (1.0°C/day) to the desired 19°C, 23°C, or 27°C. The water temperature was then maintained at the desired treatment temperatures (±1.0°C) throughout the remainder of the 90 d experiment. After acclimatization, 1080 healthy juvenile hybrid abalones (initial body weight: 1.47 ± 0.03 g; initial shell length: 20.73 ± 0.16 mm) were selected and randomly

Table 1: Formulation and proximate composition of the experimental diet (g/kg).

Ingredients (g/kg)	Dietary protein levels (g crude protein/kg diet)					
	150	200	250	300	350	400
Fish meal	10.0	65.0	120.0	175.0	230.0	285.0
Wheat gluten	30.0	40.0	50.0	60.0	70.0	80.0
Soybean meal	150.0	160.0	170.0	180.0	190.0	200.0
Kelp meal	300.0	300.0	300.0	300.0	300.0	300.0
Corn starch	440.3	370.7	301.2	231.6	162.1	92.6
Soybean lecithin	33.7	28.3	22.8	17.4	11.9	6.4
Choline chloride	5.0	5.0	5.0	5.0	5.0	5.0
$Ca(H_2PO_4)_2$	10.0	10.0	10.0	10.0	10.0	10.0
Ethoxyquin	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin mix [a]	10.0	10.0	10.0	10.0	10.0	10.0
Mineral mix [b]	10.0	10.0	10.0	10.0	10.0	10.0
Total (g)	1000	1000	1000	1000	1000	1000
Proximate composition (g/kg diet)						
Crude protein	152.5	202.5	252.6	302.6	352.7	402.7
Crude lipid	39.5	39.7	39.1	39.7	39.7	39.4
Crude ash	97.4	108.4	119.4	130.4	141.5	152.5
Carbohydrate (reducing sugar) [c]	444.8	391.6	334.5	277.3	220.1	183.0
Gross energy (kJ/g)	16.81	16.69	16.58	16.46	16.35	16.23

[a]Vitamin mix (IU or mg/kg diet): vitamin A 100,000 IU; vitamin D3 320,000 IU; vitamin E 4,600 IU; vitamin K, 1,000 mg; biotin, 8 mg; folic acid 400 mg; vitamin B1 1,500 mg; niacin, 800 mg; inositol, 12,800 mg; calcium pantothenate, 2,000 mg; vitamin B2, 2,800 mg; vitamin B6, 1,000 mg, vitamin B12, 0.18 mg. All ingredients were diluted with corn starch to 1 kg. [b]Mineral mix (mg/kg diet): NaCl, 400; $MgSO_4 \cdot 7H_2O$, 6,000; $NaH_2PO_4 \cdot 2H_2O$, 10,000; KH_2PO_4, 12,800; $FeSO_4$, 1,000; $ZnSO_4 \cdot 7H_2O$,360,000; $CoCl_2 \cdot 6H_2O$, 2; KI, 5.4; $MnSO_4 \cdot H_2O$, 120; $CuSO_4 \cdot 5H_2O$, 96; $Na_2SeO_3 \cdot 5H_2O$, 3. All ingredients were diluted with corn starch to 1 kg. [c]Carbohydrate (reducing sugar) was determined by the 3,5-dinitrosalicylic acid method [36].

assigned to 54 rectangular black cages (43 cm long × 34 cm wide × 15 cm deep; these also served as a shelter, with a density of 60 abalones per cage) and then placed into corresponding seawater temperature controlling systems. Corresponding diets were hand-fed to abalone at a rate equaling 3–5% of wet body weight once daily at 16 : 00. These rations were in excess of the abalones' daily requirements. The rations were adjusted based on the biomass at stocking and from monthly weight checks for all treatments. Feces and uneaten diets were removed at 8 : 00 every morning to maintain water quality. The experiment was subjected to a natural photoperiod of 12 : 12 h (L:D). During the feeding trial, 1/2 of the water was replaced every day, and the dissolved oxygen saturation, salinity, and pH of the water were: >90%, 30–32‰, and 7.41–7.93, respectively. All water parameters were monitored daily using a handheld water quality meter (86031 AZ Waterproof IP67 Combo Water Quality Tester) for temperature, pH, dissolved oxygen, and salinity.

2.4. Sampling. After the feeding trial, all experimental abalones were starved for 48 h. Then, abalones from each cage were counted, measured, and weighed to calculate the survival rate, increase in shell length, and weight gain. Six abalones with similar weights were randomly selected from each cage and shucked and then frozen at −20°C for whole-body composition analysis. Another 12 abalone from each tank were used to collected approximately 1 mL of hemolymph using a sterilized syringe from the blood sinus in the foot muscle, and the hemolymph was collected within 0.5 min after abalones were removed from the cages. The sample was then immediately centrifuged at 3000 × g for 10 min to collect serum and stored at −80°C for later analysis. Subsequently, these 12 abalones were shucked to separate the digestive gland. Digestive gland samples were immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis.

2.5. Proximate Composition Analysis. The proximate composition of the diets and whole body of the experimental abalone were evaluated according to the standard procedure published in AOAC [35]. In brief, moisture was determined with the drying method at 105°C, crude protein (nitrogen ×6.25) was analysed by the Kjeldahl method after acid digestion, crude lipid was determined by Soxhlet extraction, and ash was determined after combustion at 550°C to a constant weight. Carbohydrate (reducing sugar) was determined by the 3,5-dinitrosalicylic acid method [36]. Gross energy was determined using the Parr 6100 Automatic Bomb Calorimeter.

2.6. Serum Biochemical Parameters. Assays of serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin (ALB), total protein (TP), glucose (Glu), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and total cholesterol (TC), were all measured using a commercial assay kit (Mindray Shenzhen Biotechnology Co., Ltd., China).

2.7. Antioxidation Enzyme Activity. The thawed digestive gland tissue plus a 9-fold volume (v/w) of ice-cold physiological saline solution were added to a 5 ml test tube, homogenized using an IKA miniature homogenizer (T10B, IKA Co., Germany), and the homogenate was centrifuged at 10,000 × g for 20 min at 4°C. The supernatant was collected and stored at −20°C for enzyme activity determination. The Coomassie Brilliant Blue method was used to measure the protein contents contained in the above enzyme fluid. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), and glutathione peroxidase (GPx); the level of total antioxidant capacity (T-AOC); and the content of protein carbonyl (PC) and malondialdehyde (MDA) were detected according to the instructions of a matched test kit provided by the Nanjing Jiancheng Bioengineering Institute.

2.8. Assay of Reactive Oxygen Species (ROS). The levels of reactive oxygen species (ROS) in the tissue were determined by DCFH oxidation method by Keston et al. [37] with small modifications. The supernatant was incubated with 5 mM DCFH-DA (2′,7′-dichlorofluorescein diacetate) in a final volume of 2 mL Tris–HCl for 30 min at room temperature. The DCFH-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. Fluorescence was measured using excitation and emission wavelengths of 485 and 538 nm, respectively. A calibration curve was established with standard DCF (0.1 nm to 1 μm), and results were expressed as nmol of DCF/mg protein.

2.9. Date Calculation and Statistical Analysis. The data on the leaching loss rate, weight growth rate (WGR), specific growth rate (SGR), daily increment in shell length (DISL), and survival rate (SR) were calculated as follows:

$$\begin{aligned}
\mathrm{WGR}(\%) &= 100 \times \frac{[\mathrm{FW}(g/\text{abalone}) - \mathrm{IW}(g/\text{abalone})]}{\mathrm{IW}(g/\text{abalone})},\\
\mathrm{SGR}\left(\%\text{body weight}\frac{\text{gain}}{d}\right) &= 100 \times \frac{[\ln(\mathrm{FW}) - \ln(\mathrm{IW})]}{d},\\
\mathrm{DISL}\left(\frac{\text{mm}}{\text{day}}\right) &= \frac{\mathrm{FSL} - \mathrm{ISL}}{d},
\end{aligned} \tag{2}$$

where FW is final weight, IW is initial weight, ISL is initial shell length, and FSL is final shell length.

$$\begin{aligned}
\mathrm{SR}(\%) &= 100 \times \frac{\text{the final number of abalone}}{\text{the initial number of abalone}},\\
\text{The leaching loss rate}(\%) &= \frac{[(L_0 - L_t)]}{L_0} \times 100,
\end{aligned} \tag{3}$$

where L_0 is the initial weight of feed and L_t is the final weight (12 h or 24 h) of the feed.

All statistical analyses were performed using SPSS software (version 21.0). Levene's test for equality of variances was performed to ensure homogeneity of variances. One- and two-way ANOVA were used for analyzing the individual effects of dietary protein level and water temperature and the interaction between them. When overall differences were significant ($P < 0.05$), Duncan's test was used to compare the mean values among different groups. Data are presented as means ± standard deviation (SD). Quadratic regression was employed to determine the optimal dietary protein level for hybrid abalone under the three temperatures.

3. Results

3.1. Leaching Loss Rate. The leaching loss rates of the experimental diets at 12 h and 24 h under three temperatures are presented in Table 2. The leaching loss rate increased with increasing water temperature and with increasing submersion time. There was a highly significant effect of water temperature on the leaching loss rate of diets ($P < 0.001$). A lower leaching loss rate was observed at 19°C (7.80 ± 0.40% at 12 h; 11.36 ± 0.95% at 24 h) compared with 23°C (11.41 ± 0.70% at 12 h; 13.35 ± 0.84% at 24 h) and 27°C (12.62 ± 0.69% at 12 h; 17.33 ± 0.53% at 24 h). There was no significant effect of protein level on the leaching loss rate of the diets ($P > 0.05$), and there was also no significant interaction between water temperature and dietary protein level ($P > 0.05$).

3.2. Growth Performance. Data on growth performance are shown in Table 3. No dead abalone was observed in the groups reared in 19°C and 23°C. A survival rate of abalone during the feeding trial less than 100% only occurred in the 152.5 g/kg protein group (95.00 ± 2.89%), 352.7 g/kg protein group (99.44 ± 0.96%), and the 402.7 g/kg protein group (96.11 ± 1.92%) at 27°C. The survival rate of abalone was significantly affected by water temperature ($P < 0.001$), protein level ($P < 0.05$), and the interaction between these two factors ($P < 0.05$).

The abalone growth performance indices, including FW, FSL, WGR, SGR, and DISL, were significantly influenced by dietary protein level and water temperature ($P < 0.001$), but no significant ($P > 0.05$) interaction was found except for with DISL. Without consideration of dietary protein level, the FSL, WGR, and DISL of abalone significantly increased with lowering the water temperature from 27°C to 23°C and then remained stable with further lowering of the water temperature ($P < 0.05$). However, FW and SGR increased and then decreased gradually as the water temperature increased to 23°C. Disregarding the water temperature, the FW, FSL, WGR, SGR, and DISL all increased with increasing dietary protein levels and reached a peak at the 302.6 g/kg protein group. These factors all decreased gradually as the protein levels continued to increase.

A second-order polynomial regression analysis based on SGR suggested that the optimum dietary protein levels for juvenile hybrid abalone were 333.1, 318.6, and 306.3 g crude protein/kg diet at 19°C, 23°C, and 27°C, respectively

Table 2: The leaching loss rate of experimental diets at different immersing time under three water temperatures.

Water temperature (°C)	Protein level (g/kg)	Immersion for 12 h (%)	Immersion for 24 h (%)
19 °C	150	7.52 ± 0.51	10.50 ± 0.37
	200	7.80 ± 0.43	11.19 ±1.06
	250	7.57 ± 0.13	11.09 ± 1.65
	300	7.91 ± 0.56	11.60 ± 0.60
	350	8.00 ± 0.30	11.87 ± 0.53
	400	8.04 ± 0.39	11.96 ± 0.93
23°C	150	11.13 ±0.99	13.41 ± 0.57
	200	11.28 ±1.02	13.80 ± 0.76
	250	11.79 ± 0.44	13.21 ± 1.39
	300	11.30 ± 0.47	13.01 ± 0.82
	350	11.75 ± 0.47	13.09 ± 1.37
	400	11.21 ± 0.98	13.61 ± 0.26
27°C	150	15.89 ± 1.49	17.29 ± 0.39
	200	12.92 ± 0.63	17.14 ± 1.12
	250	13.10 ± 0.80	17.46 ± 0.29
	300	12.53 ±1.01	17.23±0.49
	350	12.56 ± 0.47	17.63 ± 0.20
	400	12.58 ± 0.70	17.27±0.69
Water temperature (°C)			
19 °C		7.80 ± 0.40[a]	11.36 ± 0.95[a]
23°C		11.41 ± 0.70[b]	13.35 ± 0.84[b]
27°C		12.62 ±0.69[c]	17.33 ± 0.53[c]
Protein level (g/kg)			
150		10.23±2.17	13.73 ± 2.97
200		10.66 ± 2.35	14.04 ± 2.72
250		10.81 ± 2.54	13.91 ± 3.01
300		10.57 ± 2.16	13.94 ± 2.59
350		10.76 ± 2.14	14.19 ± 2.72
400		10.61 ± 2.11	14.27±2.42
Two-way ANOVA (p value)			
Water temperature		0.000	0.000
Protein level		0.500	0.785
Interactions		0.908	0.768

Note: values are means ± SD ($n = 3$), and different superscripts in the same row are significantly different ($P < 0.05$).

(Figure 1(a)). Quadratic regressions, based on WGR, showed that the optimum dietary protein level for juvenile hybrid abalone was 310.9, 335.9, and 335.8 g crude protein/kg diet at 19°C, 23°C, and 27°C, respectively (Figure 1(b)).

3.3. Body Composition. The results of the body composition are presented in Table 4. There were no significant effects of water temperature, protein level, and interaction between the two factors on the crude lipid and ash ($P > 0.05$). Moisture decreased significantly with increasing water temperature ($P < 0.001$), but the crude protein in the body of abalone increased with increasing water temperature. Without considering water temperature, 302.6 and 352.7 g/kg protein groups significantly increased the moisture compared with other groups ($P < 0.05$), and crude protein content increased with increasing dietary protein levels ($P < 0.05$). However, we observed no significant interaction between water temperature and dietary protein on the crude protein and moisture content ($P > 0.05$).

3.4. Serum Biochemical Indices. Table 5 shows the results of serum biochemical indices of abalone. Irrespective of dietary protein levels, ALT, AST, ALP activities, and TG content in serum were significantly ($P < 0.05$) elevated at increasing water temperatures, whereas the opposite was true for ALB activity, contents of TP and Glu. However, TC content was not affected by water temperature. Disregarding water temperature, TG and TC contents both decreased significantly ($P < 0.05$) with increasing dietary protein levels, whereas the opposite was true for TP content. Other serum biochemical indices were not affected by the dietary protein levels. In addition, significant interactions between dietary protein levels and water temperature were observed in serum biochemical indices only for ALB activity and contents of TP and TG ($P < 0.05$). Dietary protein levels or water temperature exerted no significant effect on contents of HDL-C and LDL-C ($P > 0.05$).

3.5. Antioxidation Parameters. Table 6 shows that dietary protein levels had no significant ($P > 0.05$) effects on any of the digestive gland antioxidation parameters except for the value of T-AOC. However, ROS value and the content of PC and MDA first decreased and then gradually increased with increasing dietary protein levels, whereas the activities of SOD, CAT, GST, and GPx appeared to show the opposite trend. Disregarding dietary protein levels, ROS value and GST activity increased ($P < 0.05$) with increasing water temperature from 23°C to 27°C, whereas the opposite was true for the T-AOC value, CAT, and GPx activities. In addition, PC and MDA contents significantly decreased with increasing water temperature up to 23°C and then significantly increased ($P < 0.05$), but SOD showed the opposite trend. In addition, significant interaction between dietary protein levels and water temperature was observed in all the antioxidation parameters except for MDA content.

4. Discussion

4.1. Optimal Dietary Protein Level for Growth under Three Temperatures. In this study, no dead abalone was observed at 19°C and 23°C during the feeding trial, and 27°C had a high (98.05%) survival rate. The hybrid abalone exhibited high temperature tolerance, suggesting that the hybrid abalone may be a superior variety for culture in southern China. An optimum level of protein in diets is important for the growth and health of aquatic animals [38]. The optimum dietary protein level for aquatic animals is linked to the temperature of the rearing water [39]. Water temperature has a potent influence on growth performance and protein synthesis in poikilotherms such as abalone [40, 41]. Therefore, understanding the interactions between water temperature

Table 3: Growth performance of juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ fed with graded protein levels diets under three temperatures.

Water temperature (°C)	Protein level (g/kg)	Initial weight (g)	Initial shell length (mm)	Final weight (g)	Final shell length (mm)	Weight gain rate (%)	Specific growth rate (%)	Daily increment in shell length (mm/day)	Survival rate (%)
19°C	150	1.43 ± 0.05	20.52 ± 0.23	7.74 ± 0.33^{d}	38.04 ± 0.40^{d}	440.50 ± 30.29^{de}	1.87 ± 0.06^{cd}	0.18 ±0.00^{c}	100.00 ±0.00^{b}
	200	1.50 ± 0.05	20.44 ± 0.24	8.60 ± 0.22^{e}	39.40 ± 0.56^{e}	473.40 ± 17.32^{def}	1.94 ± 0.03^{cde}	0.21 ±0.01^{d}	100.00 ±0.00^{b}
	250	1.49 ± 0.03	20.51 ± 0.38	9.21 ± 0.26^{f}	40.81 ± 0.84^{f}	518.42 ± 20.87^{efgh}	2.02 ±0.04^{def}	0.22 ±0.01^{de}	100.00 ±0.00^{b}
	300	1.48 ± 0.16	20.62 ± 0.23	10.71 ± 0.60^{gh}	44.38 ± 1.56^{i}	632.19 ± 99.37^{ij}	2.21 ±0.15^{gh}	0.26 ± 0.02^{h}	100.00 ±0.00^{b}
	350	1.44 ± 0.10	20.72 ± 0.31	10.07 ± 0.25^{g}	42.83 ± 0.79^{h}	599.49 ± 37.21^{j}	2.16 ± 0.06^{fgh}	0.24 ±0.01^{fg}	100.00 ±0.00^{b}
	400	1.44 ± 0.14	20.26 ± 0.24	9.33 ± 0.24^{f}	40.97 ± 0.36^{f}	550.50 ± 44.21^{fghi}	2.08 ±0.08^{efg}	0.23±0.00^{efg}	100.00 ±0.00^{b}
23°C	150	1.51 ± 0.10	20.55 ± 0.31	8.83 ± 0.31^{ef}	39.33 ± 0.56^{e}	487.36 ± 60.42^{defg}	1.96 ± 0.11^{cde}	0.21 ±0.01^{d}	100.00 ±0.00^{b}
	200	1.47 ± 0.11	20.68 ± 0.40	9.40 ± 0.14^{f}	40.46 ± 0.43^{ef}	543.35 ± 46.77^{fghi}	2.07 ±0.08^{efg}	0.22 ±0.00^{de}	100.00 ±0.00^{b}
	250	1.51 ± 0.09	20.40 ±0.04	10.11 ± 0.58^{g}	41.17 ± 0.40^{f}	569.72 ± 77.80^{ghi}	2.11 ± 0.13^{efg}	0.23±0.00^{efg}	100.00 ±0.00^{b}
	300	1.40 ± 0.13	20.36 ± 0.21	10.88 ± 0.62^{h}	43.63 ± 0.29^{hi}	681.17 ± 78.76^{h}	2.28 ± 0.11^{h}	0.26 ±0.00^{h}	100.00 ±0.00^{b}
	350	1.45 ± 0.11	20.54 ± 0.37	10.47 ±0.15^{gh}	42.46 ± 0.41^{gh}	621.59 ± 58.38^{ij}	2.19 ± 0.09^{fgh}	0.24 ±0.00^{g}	100.00 ±0.00^{b}
	400	1.41 ± 0.07	20.58 ± 0.32	10.10 ±0.15^{g}	41.56 ± 0.22^{fg}	618.11 ± 47.35^{ij}	2.19 ± 0.07^{fgh}	0.23±0.00^{ef}	100.00 ±0.00^{b}
27°C	150	1.55 ± 0.05	20.42 ± 0.36	4.66 ± 0.39^{a}	32.24 ± 0.83^{a}	200.66 ± 30.43^{a}	1.22 ± 0.12^{a}	0.13 ±0.01^{a}	95.00 ± 2.89^{a}
	200	1.45 ± 0.11	20.64 ± 0.27	5.45 ± 0.19^{b}	33.62 ± 0.25^{b}	277.91 ± 34.87^{ab}	1.47 ± 0.10^{b}	0.14 ±0.00^{a}	100.00 ±0.00^{b}
	250	1.50 ± 0.02	20.49 ± 0.45	6.26 ±0.08^{c}	34.97 ± 0.80^{c}	315.63 ± 10.21^{b}	1.58 ± 0.03^{b}	0.16 ±0.01^{b}	100.00 ±0.00^{b}
	300	1.45 ±0.08	20.28 ± 0.10	7.46 ± 0.40^{d}	37.49 ± 0.33^{d}	413.64 ± 42.57^{cd}	1.82 ± 0.09^{c}	0.18 ±0.00^{c}	100.00 ±0.00^{b}
	350	1.51 ± 0.05	20.59 ± 0.41	6.60 ± 0.35^{c}	35.23±0.99^{c}	336.56 ± 37.91^{bc}	1.63 ± 0.10^{b}	0.16 ±0.01^{b}	99.44 ± 0.96^{b}
	400	1.49 ± 0.11	20.57 ± 0.11	6.13 ± 0.33^{c}	33.59 ± 0.91^{b}	311.24 ± 27.21^{b}	1.57 ±0.08^{b}	0.14 ±0.01^{a}	96.11 ± 1.92^{a}
Water temperature (°C)	19 °C	1.46 ±0.08	20.51 ± 0.26	9.28 ±1.03^{b}	41.07 ± 2.26^{b}	535.75 ± 80.53^{b}	2.05 ± 0.14^{b}	0.22 ± 0.02^{b}	100.00 ±0.00^{b}
	23°C	1.46 ± 0.09	20.52 ± 0.27	9.97 ± 0.76^{c}	41.44 ± 1.45^{b}	586.88 ± 82.79^{b}	2.13 ± 0.14^{c}	0.23±0.01^{b}	100.00 ±0.00^{b}
	27°C	1.49 ± 0.07	20.50 ± 0.29	6.10 ± 0.94^{a}	34.52 ± 1.81^{a}	309.27±71.06^{a}	1.55 ± 0.20^{a}	0.15 ±0.01^{a}	98.05 ± 2.69^{a}
Protein level (g/kg)	150	1.49 ±0.08	20.49 ± 0.27	7.08 ± 1.89^{a}	36.54 ± 3.32^{a}	376.17 ± 138.25^{a}	1.69 ± 0.36^{a}	0.17 ± 0.03^{a}	98.33 ± 2.88
	200	1.47 ±0.08	20.58 ± 0.29	7.82 ± 1.81^{ab}	37.83 ± 3.21^{ab}	431.55 ± 122.97^{ab}	1.83 ± 0.28^{ab}	0.19 ± 0.03^{a}	100.00 ±0.00
	250	1.50 ± 0.05	20.47 ± 0.30	8.53 ± 1.77^{ab}	38.98 ± 3.08^{abc}	467.92 ± 123.24^{abc}	1.90 ± 0.25^{ab}	0.20 ± 0.03^{ab}	100.00 ±0.00
	300	1.44 ± 0.11	20.42 ± 0.22	9.69 ± 1.73^{b}	41.83 ± 3.37^{c}	575.67 ± 140.32^{c}	2.10 ± 0.24^{b}	0.23±0.04^{ab}	100.00 ±0.00
	350	1.47 ±0.08	20.62 ± 0.31	9.05 ± 1.85^{b}	40.17 ± 3.77^{bc}	519.21 ± 142.68^{bc}	2.00 ± 0.28^{b}	0.21 ±0.04^{b}	99.81 ± 0.55
	400	1.44 ± 0.10	20.47 ± 0.26	8.52 ± 1.83^{ab}	38.71 ± 3.88^{abc}	493.28 ± 143.99^{abc}	1.95 ± 0.29^{ab}	0.20 ±0.04^{ab}	98.70 ± 2.16
Two-way ANOVA (*P* value)									
Water temperature		0.452	0.977	0.000	0.000	0.000	0.000	0.000	0.000
Protein level		0.691	0.710	0.000	0.000	0.000	0.000	0.000	0.025
Interactions		0.894	0.749	0.581	0.077	0.985	0.384	0.013	0.008

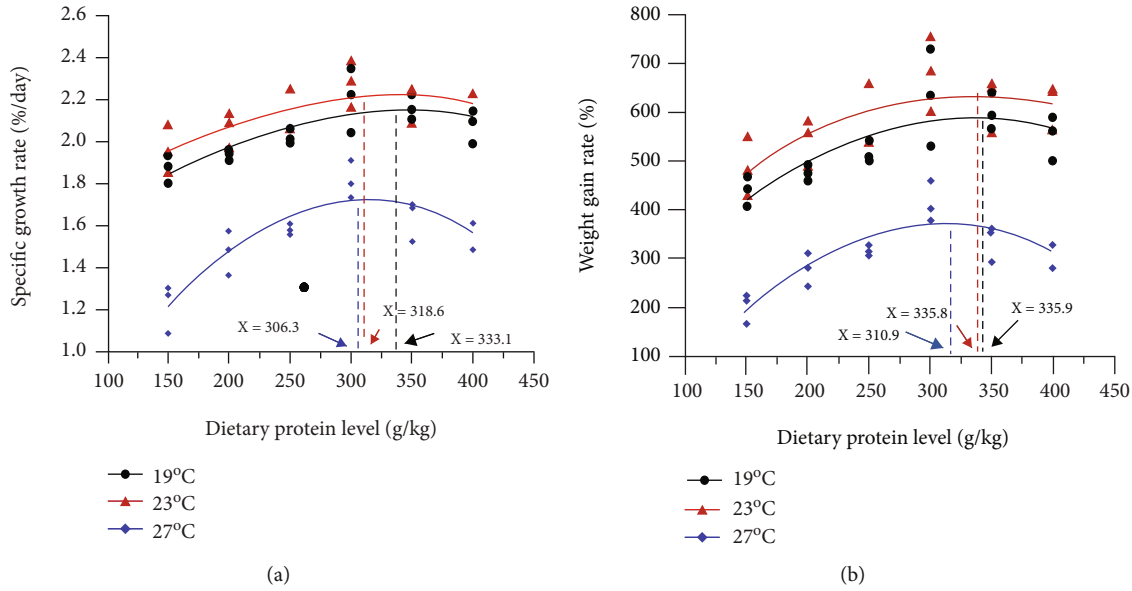

Figure 1: Notes: (a) second-order polynomial regression analysis of specific growth rate (%/day) to dietary protein levels (g/kg) in juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ reared at three temperatures (19°C: $y = -0.000009x^2 + 0.005995x + 1.141210$, $R^2 = 0.85$, $X_{max=333.1}$; 23°C: $y = -0.000008x^2 + 0.005097x + 1.355312$, $R^2 = 0.86$, $X_{max=318.6}$; 27°C: $y = -0.00002x^2 + 0.01225x - 0.18480$, $R^2 = 0.91305$, $X_{max=306.3}$). (b) Second-order polynomial regression analysis of weight gain rate (%) to dietary protein levels (g/kg) in juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ reared at three temperatures (19°C: $y = -0.0051x^2 + 3.4253x + 20.412$, $R^2 = 0.81$, $X_{max} = 335.8$; 23°C: $y = -0.0046x^2 + 3.0904x + 116.79$, $R^2 = 0.83$, $X_{max} = 335.9$; 27°C: $y = -0.0069x^2 + 4.2911x - 295.09$, $R^2 = 0.86$, $X_{max} = 310.9$).

and optimum dietary protein level is important for optimizing diets by using specific levels of dietary protein at different water temperatures throughout the culture period. In this study, we observed that FW and FSL increased with increasing dietary protein levels and reached their highest levels at 302.6 g protein/kg diet under all three water temperatures. Increasing protein level in the diet resulted in a decrease of growth performance. Disregarding the water temperature, WGR and SGR increased with increasing dietary protein levels and reached a peak at the 302.6 g/kg protein group and decreased gradually as the protein levels continued to increase. Similar results were reported for other abalone species, such as *H. discus hannai* [13, 14], *H. midue* [15, 17], *H. laevigata* [19], and *H. iris* [22]. A second-order polynomial regression analysis based on SGR suggested that the optimum dietary protein levels for juvenile DF were 333.1, 318.6, and 306.3 g crude protein/kg diet at 19°C, 23°C, and 27°C, respectively. This result was consistent with a study in which abalone (*H. iris*) grown at lower temperatures (8-16°C) required 42% protein in their diet compared to 38% needed for those grown at 13–21°C [22]. It is possible that temperature regulates protein turnover and influences the protein requirements of aquatic animals. However, our results differed from those of Stone et al. [19], who found that the optimum dietary protein levels of greenlip abalone (*H. laevigata*) increased from 29% to 35% when temperature increased from 14 to 22°C. These differences might be due to a combination of the experimental design range of water temperature, abalone size and species, feeding frequency, and feed formulation. Aquatic animal growth inhibition may be related to a number of factors, including protein deposition in the whole body. Hence, we determined the effect of water temperature and dietary protein level on the body composition of hybrid abalone.

4.2. Body Composition. The body composition of aquatic animals is affected by exogenous factors such as environment and feed composition [42]. In our research, the body protein content and moisture increased with increased dietary protein level. This result was consistent with studies on GIFT tilapia (*Oreochromis niloticus*) [30], sea bream [43], and red spotted grouper [44], demonstrating that the whole body moisture content increased with the increase of dietary protein. Similar increasing trends of body protein content were also observed in the grouper (*Epinephelus coioides*) [34], giant grouper (*Epinephelus lanceolatus*) [45], *Colossoma macropomum* (Cuvier) [46], Senegalese sole [47], bagrid catfish [48], hybrid *Clarias* catfish [49], and gilthead sea bream fry [50]. According to the previous report, the feed protein level was directly proportional to the body protein content of aquatic animals within a certain range. Higher protein diets contributed to more protein ingested, which could accelerate protein deposition in body tissues for aquatic animals, and eventually lead to the body protein content increased [51]. However, the findings of the current study disagree with those of a previous study that found no significant differences in soft body protein content among different dietary protein level treatments in black buffalo (*Ictiobus niger*) [52]. This inconsistency may be due to the relative sizes of aquatic animals. In salmonids, the protein content of growing salmonids was determined solely by fish size [53]. In this study, we found that the protein content of the soft body increased as water temperature increased. Similar results were reported by Fatma et al. [54] who found that

Table 4: The nutrition components (%, wet weight basis) of the soft body of juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ fed test diets under three temperatures.

Water temperature (°C)	Protein level (g/kg)	Moisture	Crude protein	Crude lipid	Ash
19°C	150	77.83 ± 0.91^{ef}	19.10 ± 1.03^{a}	0.97 ± 0.10	3.02 ± 0.06
	200	78.14 ± 1.07^{f}	19.32 ± 1.16^{ab}	0.97 ± 0.15	3.07 ± 0.02
	250	77.38 ± 0.38^{def}	19.86 ± 0.16^{abc}	0.90 ± 0.10	3.08 ± 0.07
	300	77.78 ± 1.12^{ef}	20.20 ± 0.98^{abcde}	0.88 ± 0.09	3.06 ± 0.08
	350	77.84 ± 1.19^{ef}	20.01 ± 0.97^{abcd}	0.99 ± 0.04	3.07 ± 0.05
	400	78.28 ± 0.98^{f}	20.59 ± 0.28^{bcdef}	0.98 ± 0.13	3.02 ± 0.04
23°C	150	76.83 ± 0.33^{cdef}	19.56 ± 0.68^{abc}	1.01 ± 0.15	3.02 ± 0.09
	200	75.41 ± 0.76^{abcd}	19.79 ± 0.25^{abc}	0.94 ± 0.05	3.07 ± 0.11
	250	75.55 ± 1.23^{bcd}	19.95 ± 0.27^{abcd}	0.99 ± 0.12	3.02 ± 0.06
	300	75.37 ± 1.01^{abcd}	19.98 ± 0.32^{abcd}	1.00 ± 0.01	3.00 ± 0.08
	350	75.90 ± 0.93^{bcde}	20.32 ± 0.52^{abcde}	0.99 ± 0.06	3.05 ± 0.07
	400	75.57 ± 1.09^{bcd}	20.87 ± 0.09^{cdef}	1.01 ± 0.02	3.03 ± 0.09
27°C	150	74.02 ± 0.85^{ab}	19.92 ± 0.50^{abcd}	1.01 ± 0.02	3.07 ± 0.05
	200	75.02 ± 1.81^{abc}	19.53 ± 0.58^{ab}	1.02 ± 0.11	3.03 ± 0.05
	250	74.45 ± 1.36^{ab}	19.96 ± 1.31^{abcd}	1.01 ± 0.12	3.06 ± 0.08
	300	74.21 ± 1.99^{ab}	21.22 ± 0.61^{def}	0.99 ± 0.03	3.02 ± 0.02
	350	73.93 ± 1.30^{ab}	21.59 ± 0.15^{f}	1.04 ± 0.16	3.03 ± 0.03
	400	73.26 ± 0.97^{a}	21.34 ± 0.41^{ef}	1.01 ± 0.02	3.07 ± 0.05
Water temperature (°C)	19 °C	77.87 ± 0.87^{c}	19.85 ± 0.89^{a}	0.95 ± 0.10	3.05 ± 0.05
	23 °C	75.77 ± 0.94^{b}	20.08 ± 0.55^{ab}	0.99 ± 0.08	3.03 ± 0.07
	27°C	74.15 ± 1.33^{a}	20.59 ± 1.01^{b}	1.01 ± 0.08	3.05 ± 0.05
Protein level (g/kg)	150	76.23 ± 1.83^{a}	19.53 ± 0.75^{a}	1.00 ± 0.09	3.04 ± 0.07
	200	76.19 ± 1.85^{ab}	19.55 ± 0.69^{a}	0.98 ± 0.10	3.06 ± 0.06
	250	75.80 ± 1.59^{ab}	19.92 ± 0.67^{a}	0.97 ± 0.11	3.05 ± 0.06
	300	75.78 ± 2.01^{b}	20.47 ± 0.83^{b}	0.96 ± 0.07	3.03 ± 0.07
	350	75.89 ± 1.97^{b}	20.64 ± 0.91^{b}	1.01 ± 0.09	3.05 ± 0.05
	400	75.70 ± 2.34^{ab}	20.93 ± 0.41^{c}	1.00 ± 0.07	3.04 ± 0.06
Two-way ANOVA (P value)					
Water temperature		0.000	0.007	0.160	0.638
Protein level		0.884	0.000	0.835	0.941
Interactions		0.733	0.602	0.982	0.872

Note: values are means ± SD ($n = 3$), and different superscripts in the same row are significantly different ($P < 0.05$).

the protein content of the *Heteropneustes fossilis* body showed a positive correlation with water temperatures from 18°C to 26°C. However, our outcome was in contrast to that reported in leopard coral grouper [55] and minnow [56]. This result might have resulted from high water temperature, which reduced the feed intake. A greater proportion of nutrients was used for increased metabolism, which resulted in a decline of protein content [57]. The variability in the reported results may be due to differences of optimum water temperature for different aquatic species. Further research on the specific mechanism of water temperature regulating the body composition of aquatic species is needed.

4.3. Serum Biochemistry. Serum biochemistry indicators are often used to evaluate the health status of aquatic animals and their adaptability to the environment [28]. These indicators also reflect the metabolism and physiological status of aquatic animals [58]. Glu, TG, and TP are the main forms of nutrients in the blood after being digested and absorbed by animals, which reflect the metabolism of carbohydrates, lipids, and proteins, respectively [59]. Glu reflects the metabolism of sugar [60], and it also reflects whether diet nutrition is appropriate and whether endocrine function is effective [61]. TG is a main source of blood lipids, which are formed by utilizing glucose and fatty acids and released into the blood [62]. The TP content in serum reflects the absorption and metabolism of protein in the body [63]. Many studies have evaluated changes in water temperature as a stressor of aquatic species and monitored its effects on serum biochemistry [64, 65]. Our study showed that TP and Glu contents in serum were significantly reduced at 27°C compared with the 19°C and 23°C groups, whereas the opposite was true for TG content, which was similar to the results of studies on *Oreochromis niloticus* [30] and spotted seabass (*Lateolabrax maculatus*) [10]. In addition, dietary nutritional level,

Table 5: Plasma haematological parameters of juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ fed with diets containing different protein levels under three temperatures.

Water temperature (°C)	Protein level (g/kg)	ALT (U/L)	AST (U/L)	ALP (U/L)	ALB (U/L)	TP (g/L)	Glu (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)	TC (mmol/L)
19 °C	150	14.00 ± 0.61^{a}	160.30 ± 6.67^{gh}	1.62 ± 0.24^{bc}	5.22 ± 0.07^{d}	24.20 ± 0.61^{de}	3.68 ± 0.36^{c}	0.35 ± 0.04	0.25 ± 0.06	0.37 ± 0.02^{f}	5.60 ± 0.20^{f}
	200	11.80 ± 2.50^{a}	144.87 ± 11.21^{efgh}	1.62 ± 0.08^{bc}	5.33 ± 0.07^{de}	24.77 ± 0.93^{de}	2.62 ± 0.19^{b}	0.28 ± 0.05	0.28 ± 0.04	0.35 ± 0.02^{f}	5.43 ± 0.15^{ef}
	250	11.30 ± 3.67^{a}	140.37 ± 8.50^{defg}	1.50 ± 0.19^{abc}	5.42 ± 0.29^{de}	23.90 ± 1.51^{cd}	2.45 ± 0.35^{ab}	0.31 ± 0.06	0.27 ± 0.08	0.32 ± 0.02^{ef}	4.77 ± 0.25^{bcd}
	300	11.33 ± 1.26^{a}	117.00 ± 7.75^{abc}	1.34 ± 0.23^{abc}	5.71 ± 0.24^{e}	23.63 ± 1.50^{cd}	2.61 ± 0.27^{b}	0.23 ± 0.12	0.31 ± 0.03	0.31 ± 0.12^{def}	4.60 ± 0.17^{bc}
	350	11.13 ± 2.18^{a}	104.27 ± 5.88^{a}	1.32 ± 0.14^{abc}	5.61 ± 0.26^{de}	24.23 ± 1.33^{de}	2.41 ± 0.15^{ab}	0.18 ± 0.11	0.29 ± 0.06	0.24 ± 0.04^{abcd}	4.50 ± 0.17^{bc}
	400	12.50 ± 1.74^{a}	107.43 ± 4.99^{ab}	1.26 ± 0.12^{ab}	5.64 ± 0.42^{de}	24.20 ± 1.57^{de}	2.30 ± 0.26^{ab}	0.26 ± 0.12	0.32 ± 0.09	0.18 ± 0.02^{a}	3.43 ± 0.15^{a}
23 °C	150	59.77 ± 2.24^{c}	166.63 ± 11.63^{h}	1.65 ± 0.15^{c}	5.28 ± 0.13^{de}	22.33 ± 0.21^{c}	3.69 ± 0.21^{c}	0.36 ± 0.07	0.30 ± 0.07	0.36 ± 0.02^{f}	5.23 ± 0.21^{def}
	200	50.60 ± 2.95^{b}	146.77 ± 7.07^{fgh}	1.49 ± 0.31^{abc}	5.31 ± 0.45^{de}	23.97 ± 1.55^{cd}	2.71 ± 0.26^{b}	0.24 ± 0.05	0.31 ± 0.06	0.32 ± 0.03^{ef}	5.33 ± 0.31^{ef}
	250	50.63 ± 0.78^{b}	145.53 ± 15.28^{fgh}	1.32 ± 0.26^{abc}	5.29 ± 0.29^{de}	24.47 ± 0.45^{de}	2.43 ± 0.20^{ab}	0.25 ± 0.06	0.33 ± 0.05	0.24 ± 0.01^{abc}	5.37 ± 0.31^{ef}
	300	50.23 ± 6.35^{b}	130.33 ± 17.37^{cdef}	1.40 ± 0.12^{abc}	5.44 ± 0.29^{de}	24.40 ± 0.95^{de}	2.35 ± 0.48^{ab}	0.31 ± 0.16	0.29 ± 0.04	0.22 ± 0.01^{ab}	4.27 ± 0.15^{b}
	350	50.43 ± 4.70^{b}	125.07 ± 12.51^{bcde}	1.37 ± 0.17^{abc}	5.46 ± 0.25^{de}	24.77 ± 0.81^{de}	2.53 ± 0.40^{b}	0.31 ± 0.03	0.31 ± 0.05	0.23 ± 0.02^{ab}	4.27 ± 0.40^{b}
	400	50.37 ± 5.42^{b}	121.90 ± 9.50^{abcd}	1.19 ± 0.06^{a}	5.70 ± 0.17^{e}	25.80 ± 0.17^{e}	2.46 ± 0.32^{ab}	0.21 ± 0.03	0.25 ± 0.04	0.24 ± 0.06^{abcd}	3.50 ± 0.10^{a}
27 °C	150	82.30 ± 3.01^{f}	384.70 ± 10.83^{k}	2.75 ± 0.10^{e}	1.25 ± 0.10^{a}	10.53 ± 0.38^{a}	2.64 ± 0.18^{b}	0.20 ± 0.08	0.30 ± 0.06	0.36 ± 0.02^{f}	5.33 ± 0.25^{ef}
	200	75.67 ± 1.21^{e}	383.67 ± 7.81^{k}	2.34 ± 0.27^{d}	1.94 ± 0.12^{b}	10.40 ± 0.17^{a}	1.99 ± 0.18^{b}	0.22 ± 0.11	0.30 ± 0.08	0.35 ± 0.03^{f}	5.33 ± 0.35^{ef}
	250	67.07 ± 4.29^{d}	370.27 ± 4.45^{jk}	2.40 ± 0.25^{d}	1.91 ± 0.06^{b}	12.57 ± 0.98^{b}	1.42 ± 0.13^{a}	0.26 ± 0.12	0.28 ± 0.06	0.31 ± 0.02^{def}	5.47 ± 0.35^{ef}
	300	66.47 ± 4.55^{d}	361.07 ± 13.87^{ij}	2.31 ± 0.29^{d}	1.97 ± 0.13^{b}	12.13 ± 0.59^{b}	1.45 ± 0.18^{a}	0.25 ± 0.11	0.29 ± 0.08	0.30 ± 0.01^{cdef}	4.93 ± 0.57^{cde}
	350	68.53 ± 4.52^{d}	376.97 ± 18.34^{jk}	2.20 ± 0.13^{d}	2.00 ± 0.09^{b}	13.20 ± 0.17^{b}	1.39 ± 0.09^{a}	0.27 ± 0.11	0.25 ± 0.04	9.05 ± 1.85^{d}	4.60 ± 0.36^{bc}
	400	69.40 ± 3.61^{d}	344.37 ± 12.54^{i}	2.23 ± 0.19^{d}	2.77 ± 0.21^{c}	13.20 ± 0.10^{b}	1.38 ± 0.10^{a}	0.22 ± 0.13	0.33 ± 0.06	0.27 ± 0.02^{bcde}	3.53 ± 0.21^{a}
Water temperature (°C)	19 °C	12.01 ± 2.13^{a}	129.04 ± 22.32^{a}	1.44 ± 0.21^{a}	5.49 ± 0.28^{b}	24.16 ± 1.14^{b}	2.68 ± 0.53^{b}	0.27 ± 0.10	0.29 ± 0.06	0.29 ± 0.08^{a}	4.72 ± 0.74
	23°C	52.01 ± 5.03^{b}	138.87 ± 18.39^{a}	1.40 ± 0.22^{a}	5.41 ± 0.28^{b}	24.29 ± 1.28^{b}	2.70 ± 0.55^{b}	0.28 ± 0.08	0.30 ± 0.05	0.27 ± 0.06^{ab}	4.66 ± 0.75
	27°C	71.57 ± 6.61^{c}	370.17 ± 17.71^{b}	2.37 ± 0.26^{b}	1.97 ± 0.46^{a}	12.01 ± 1.26^{a}	1.71 ± 0.49^{a}	0.24 ± 0.10	0.29 ± 0.06	0.32 ± 0.03^{b}	4.87 ± 0.75
Protein level (g/kg)	150	52.02 ± 30.20	236.21 ± 111.71	2.01 ± 0.58	3.92 ± 2.00	19.02 ± 6.43	3.34 ± 0.57^{a}	0.30 ± 0.10	0.28 ± 0.06	0.36 ± 0.01^{c}	5.39 ± 0.25^{c}
	200	46.02 ± 27.94	225.10 ± 119.18	1.82 ± 0.45	4.19 ± 1.71	19.71 ± 7.05	2.44 ± 0.39^{b}	0.25 ± 0.07	0.29 ± 0.05	0.34 ± 0.03^{c}	5.37 ± 0.25^{c}
	250	43.00 ± 24.98	218.72 ± 114.04	1.74 ± 0.54	4.21 ± 1.74	20.31 ± 5.89	2.10 ± 0.55^{b}	0.27 ± 0.08	0.29 ± 0.06	0.29 ± 0.04^{b}	5.20 ± 0.42^{c}
	300	42.68 ± 24.85	202.80 ± 119.42	1.68 ± 0.51	4.37 ± 1.82	20.06 ± 6.02	2.14 ± 0.60^{b}	0.26 ± 0.12	0.30 ± 0.05	0.28 ± 0.08^{b}	4.60 ± 0.42^{b}
	350	43.37 ± 25.65	202.10 ± 131.96	1.63 ± 0.45	4.35 ± 1.78	20.73 ± 5.71	2.11 ± 0.58^{b}	0.25 ± 0.10	0.28 ± 0.05	0.26 ± 0.04^{ab}	4.46 ± 0.32^{b}
	400	44.09 ± 25.31	191.23 ± 115.32	1.56 ± 0.51	4.70 ± 1.47	21.07 ± 5.99	2.05 ± 0.55^{b}	0.23 ± 0.09	0.30 ± 0.07	0.23 ± 0.05^{a}	3.49 ± 0.15^{a}
Two-way ANOVA (P value)											
Water temperature		0.000	0.000	0.000	0.000	0.000	0.000	0.392	0.890	0.002	0.096
Protein level		0.000	0.000	0.001	0.000	0.001	0.000	0.654	0.991	0.000	0.000
Interactions		0.061	0.070	0.856	0.017	0.025	0.811	0.476	0.672	0.041	0.072

Note: values are means ± SD, and different superscripts in the same row are significantly different ($P < 0.05$). Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; ALB: albumin; TP: total protein; Glu: glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; TC: total cholesterol.

Table 6: Antioxidant parameters in digestive gland of juvenile hybrid abalones *H. discus hannai*♀ × *H. fulgens*♂ fed diets containing different protein levels under three temperatures.

Water temperature (°C)	Protein level (g/kg)	ROS	PC	MDA	T-AOC	SOD	CAT	GST	GPx
19°C	150	100.00 ± 2.76^{d}	9.52 ± 0.41^{e}	31.56 ± 2.33^{d}	2.21 ± 0.16^{h}	1.09 ± 0.08^{c}	1.98 ± 0.10^{e}	33.39 ± 1.85^{d}	1.98 ± 0.20^{c}
	200	95.76 ± 1.62^{cd}	7.24 ± 0.43^{d}	26.15 ± 2.87^{c}	2.43 ± 0.16^{i}	1.45 ± 0.04^{de}	2.17 ± 0.28^{e}	37.02 ± 2.55^{de}	2.10 ± 0.17^{cd}
	250	87.03 ± 3.50^{b}	6.48 ± 0.65^{cd}	26.07 ± 2.76^{c}	2.70 ± 0.03^{j}	1.64 ± 0.10^{e}	2.12 ± 0.17^{e}	47.20 ± 3.26^{hi}	2.46 ± 0.01^{fg}
	300	80.08 ± 3.70^{a}	6.34 ± 0.80^{cd}	26.54 ± 1.17^{c}	2.00 ± 0.13^{g}	2.35 ± 0.20^{f}	2.44 ± 0.23^{f}	57.19 ± 2.61^{k}	2.65 ± 0.10^{g}
	350	90.57 ± 4.61^{bc}	5.96 ± 0.72^{c}	26.75 ± 3.34^{c}	1.64 ± 0.10^{de}	2.41 ± 0.11^{fg}	2.51 ± 0.08^{f}	51.66 ± 1.90^{j}	2.23 ± 0.15^{de}
	400	91.03 ± 4.52^{bc}	6.41 ± 0.30^{cd}	26.88 ± 1.99^{c}	1.41 ± 0.04^{c}	2.23 ± 0.12^{f}	2.55 ± 0.14^{f}	50.58 ± 1.09^{ij}	2.26 ± 0.16^{def}
23°C	150	94.28 ± 1.93^{c}	3.48 ± 0.20^{b}	21.79 ± 0.92^{b}	1.80 ± 0.05^{f}	2.44 ± 0.21^{f}	1.27 ± 0.08^{ab}	37.09 ± 3.14^{de}	1.91 ± 0.02^{c}
	200	87.60 ± 2.35^{b}	3.46 ± 0.41^{b}	18.22 ± 0.89^{ab}	2.19 ± 0.06^{h}	2.88 ± 0.15^{i}	1.37 ± 0.11^{bc}	47.14 ± 1.43^{hi}	1.95 ± 0.13^{c}
	250	78.42 ± 2.13^{a}	2.01 ± 0.07^{a}	17.28 ± 0.97^{a}	2.39 ± 0.03^{i}	3.59 ± 0.15^{i}	1.73 ± 0.11^{d}	45.41 ± 3.05^{gh}	2.38 ± 0.10^{ef}
	300	78.29 ± 1.58^{a}	2.07 ± 0.14^{a}	18.02 ± 0.95^{ab}	2.53 ± 0.13^{i}	2.71 ± 0.18^{hi}	1.69 ± 0.09^{d}	57.43 ± 2.05^{k}	2.46 ± 0.16^{fg}
	350	87.20 ± 1.72^{b}	1.57 ± 0.19^{a}	18.79 ± 0.63^{ab}	2.10 ± 0.09^{gh}	2.57 ± 0.19^{gh}	1.62 ± 0.09^{d}	41.70 ± 3.84^{fg}	2.12 ± 0.13^{cd}
	400	95.33 ± 1.89^{cd}	1.74 ± 0.14^{a}	18.35 ± 0.83^{b}	1.75 ± 0.05^{ef}	1.33 ± 0.11^{d}	1.58 ± 0.15^{cd}	38.15 ± 2.78^{ef}	2.10 ± 0.04^{cd}
27°C	150	108.72 ± 1.36^{e}	14.09 ± 0.56^{g}	41.35 ± 0.15^{g}	1.18 ± 0.02^{b}	1.37 ± 0.12^{d}	1.24 ± 0.03^{ab}	34.24 ± 2.60^{de}	1.39 ± 0.11^{b}
	200	110.92 ± 1.93^{e}	11.58 ± 0.21^{f}	37.84 ± 2.54^{fg}	1.36 ± 0.10^{c}	0.88 ± 0.02^{bc}	1.32 ± 0.04^{ab}	29.16 ± 2.34^{c}	1.47 ± 0.06^{b}
	250	108.59 ± 2.47^{e}	10.15 ± 0.83^{e}	33.30 ± 5.79^{de}	1.50 ± 0.12^{cd}	0.95 ± 0.06^{bc}	1.27 ± 0.08^{ab}	26.40 ± 2.03^{bc}	1.57 ± 0.01^{b}
	300	117.97 ± 1.13^{f}	11.45 ± 0.86^{f}	33.25 ± 1.87^{de}	1.12 ± 0.07^{b}	0.80 ± 0.05^{ab}	1.24 ± 0.12^{ab}	23.37 ± 1.53^{ab}	1.55 ± 0.11^{b}
	350	117.16 ± 5.19^{f}	14.36 ± 1.37^{g}	39.13 ± 2.25^{fg}	0.80 ± 0.07^{a}	0.76 ± 0.05^{ab}	1.14 ± 0.08^{ab}	23.88 ± 2.26^{ab}	1.19 ± 0.07^{a}
	400	118.26 ± 2.14^{f}	14.51 ± 0.22^{g}	36.61 ± 3.63^{ef}	0.72 ± 0.04^{a}	0.62 ± 0.04^{a}	1.08 ± 0.10^{a}	20.39 ± 1.49^{a}	1.11 ± 0.05^{a}
Water temperature (°C)	19°C	90.74 ± 7.16^{a}	6.99 ± 1.32^{b}	27.33 ± 2.89^{b}	2.07 ± 0.46^{b}	1.86 ± 0.52^{b}	2.29 ± 0.27^{c}	46.18 ± 8.82^{a}	2.28 ± 0.26^{b}
	23°C	86.85 ± 7.12^{a}	2.39 ± 0.83^{a}	18.74 ± 1.65^{a}	2.13 ± 0.30^{b}	2.59 ± 0.70^{c}	1.54 ± 0.20^{b}	44.49 ± 7.40^{a}	2.15 ± 0.23^{b}
	27°C	113.60 ± 4.96^{b}	12.69 ± 1.87^{c}	36.91 ± 4.06^{c}	1.11 ± 0.29^{a}	0.90 ± 0.25^{a}	1.22 ± 0.11^{a}	26.24 ± 4.93^{b}	1.38 ± 0.19^{a}
Protein level (g/kg)	150	101.00 ± 6.55	9.03 ± 4.62	31.57 ± 8.56	1.73 ± 0.46^{abc}	1.63 ± 0.63	1.49 ± 0.37	34.91 ± 2.79	1.76 ± 0.30
	200	98.09 ± 10.40	7.43 ± 3.53	27.40 ± 8.77	1.99 ± 0.49^{bc}	1.74 ± 0.90	1.62 ± 0.44	37.78 ± 8.03	1.84 ± 0.31
	250	91.35 ± 13.67	6.21 ± 3.57	25.55 ± 7.66	2.20 ± 0.54^{c}	2.06 ± 1.19	1.71 ± 0.38	39.67 ± 10.28	2.14 ± 0.43
	300	92.11 ± 19.52	6.62 ± 4.11	25.94 ± 6.72	1.88 ± 0.63^{bc}	1.95 ± 0.89	1.79 ± 0.54	46.00 ± 17.07	2.22 ± 0.52
	350	98.31 ± 14.66	7.30 ± 5.68	28.22 ± 9.11	1.51 ± 0.58^{ab}	1.91 ± 0.88	1.76 ± 0.60	39.08 ± 12.43	1.85 ± 0.50
	400	101.54 ± 12.96	7.55 ± 5.60	27.28 ± 8.19	1.30 ± 0.46^{a}	1.40 ± 0.70	1.74 ± 0.66	36.37 ± 13.25	1.82 ± 0.55
Two-way ANOVA (P value)									
Water temperature		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Protein level		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Interactions		0.000	0.000	0.565	0.000	0.000	0.000	0.000	0.001

Note: values are means ± SD ($n = 3$), and different superscripts in the same row are significantly different ($P < 0.05$). Abbreviations: ROS: reactive oxygen species; PC: protein carbonyl; MDA: malondialdehyde; T-AOC: total antioxidant capacity; SOD: superoxide dismutase; CAT: catalase; GST: glutathione S-transferase; GPx: glutathione peroxidase.

including protein level, can also regulate the serum biochemical indicators of aquatic animals [66, 67]. Disregarding water temperature, we found that TG and Glu contents in serum gradually decreased with increasing levels of dietary protein while TP content showed an opposite trend. Only TG content was remarkably influenced by dietary protein levels. Similar results were found in the grey mullet [68], Chinese mitten crab (*Eriocheir sinensis*) [69], and orange-spotted grouper [51]. When aquatic animals ingested low-protein diet, they also consumed high levels of carbohydrates, which provided more glucose. Glycolysis is a necessary common stage for glucose catabolism in aquatic animals, studies in mud crab S. *paramamosain* [70], and abalone (*Haliotis discus hannai*) [71] showed that blood glucose was directly proportional to dietary carbohydrate levels. Hence, we speculated that the low-protein diet could activate the pathway of glycolysis in abalone and produced more acetyl coenzyme A and dihydroxyacetone phosphate, which would further lead to production of TG by lipid synthesis.

Protein is metabolized and transformed in fish through transamination and deamination [72]. AST and ALT act to optimize growth and energy utilization by redistributing the amino groups to form new amino acids, thus indicating protein availability [73]. Research in fish indicates that ALT and AST levels in serum can be used as biomarkers of protein degradation and liver damage. The fish showed significant increase in serum ALT and AST levels suggesting protein degradation and liver damage [74]. The digestive gland (often called hepatopancreas) in mollusks also reflects some functional similarities of the organ and the vertebrate liver and pancreas [75]. It is also reported that elevated AST and ALT activities in serum represent reduced catabolism of protein and impaired digestive gland in gastropods [76]. In this study, ALT and AST activities in serum were significantly elevated in the 27°C group compared to the 19°C and 23°C groups. These data suggest that a higher water temperature reduced protein efficiency and modified digestive gland function of the hybrid abalone. A similar result was reported in tilapia [30]. Disregarding water temperature, ALT and AST activities in serum showed an increased trend with dietary protein levels reduced at the three temperature levels, respectively, which implied that reducing protein efficiency and catabolism of protein with low protein levels. When in vivo protein synthesis is insufficient, aquatic animals cannot effectively decompose protein to meet the energy requirements [77, 78].

4.4. Antioxidation Enzyme Activities. The dynamic balance of free radicals is orchestrated by continuous ROS production, but excess ROS cause oxidative stress and damage biomolecules [79]. Biomarkers of oxidative damage include PC and the lipid peroxidation product MDA [80]. In this study, the ROS, MDA, and PC levels in the digestive gland of hybrid abalone first decreased and then increased with elevated water temperature. It has been demonstrated that high temperature, like other adverse environmental conditions, is an important inducer of oxidative stress in aquatic organisms, promoting formation of ROS [81]. Our results indicated higher water temperature caused oxidative stress of the hybrid abalone. This outcome is similar to the results in oriental river prawn [82] and red claw crayfish (*Cherax quadricarinatus*) [32]. In addition, ROS, MDA, and PC contents decreased (152.5–252.6 g protein/kg groups) and thereafter decreased gradually as dietary protein levels increased. The excessive and deficient dietary protein level can break the oxidative balance of body. A previous study also reported that excessive (43.27%) and deficient (17.64%) dietary protein level could reduce the antioxidative capacity of abalone [29]. Similar results were obtained in the present study. When fed a diet deficient in protein, abalone will grow slowly, and antioxidation functions will decrease. When fed an excess of protein, the digestibility of protein will be reduced, and this will also affect the antioxidation system. Water temperature, dietary protein, and their interactions had significant effects on antioxidant enzyme activities in the digestive gland of the hybrid abalone. T-AOC is an index reflecting the overall capacity of the organism's antioxidant enzymatic system [83]. In this study, the T-AOC level in the digestive gland of abalone decreased with increasing water temperature. Abalone in the 27°C group had a significantly lower T-AOC value compared with the 19°C and 23°C groups. With dietary protein levels at 252.6 g/kg, the T-AOC value was markedly enhanced and then decreased progressively. This outcome is partly similar to the results of Zhu et al. [69], who found that the serum T-AOC level decreased with increasing dietary protein levels in crabs. SOD and CAT activities are the key enzymes involved in scavenging excess free radicals and lipid peroxides, protecting aquatic animals from oxidative damage [84]. The results of this study showed that the activities of SOD and CAT in the 19°C and 23°C groups were significantly higher than activities in the 27°C group, which indicated that a higher water temperature reduced the activities of antioxidant enzymes and caused oxidative stress. This is consistent with earlier research, which showed that SOD and CAT activities of the 25°C group were significantly higher than in the 20°C and 30°C groups in *Cherax quadricarinatus* [32]. Regardless of the water temperature, the activities of SOD and CAT were increased with dietary protein levels up to 252.6 g/kg and 302.6 g/kg, respectively, and then decreased. This result is consistent with the tendency of SOD activity in *Eriocheir sinensis* [69].

5. Summary

This study explored the optimum protein level, body composition, serum biochemistry, and digestive gland antioxidant enzyme activities of the hybrid abalone *H. discus hannai*♀ × *H. fulgens*♂ under three water temperatures. Based on SGR, the optimal dietary protein levels for juvenile hybrid abalones (initial body weight: 1.47 ± 0.03 g; initial shell length: 20.73 ± 0.16 mm) were 333.1, 318.6, and 306.3 g protein/kg diet at 19°C, 23°C, and 27°C, respectively. The body protein content increased with the increase of dietary protein levels and water temperature, but no significant interaction was observed. In addition, TP and Glu in the serum of abalone were reduced with the increase of water temperature, whereas the opposite was true for TG. TG

and Glu decreased with increasing levels of dietary protein, and TP showed an opposing trend. High water temperature and inappropriate diet protein levels both resulted in oxidative stress and a decrease of antioxidant enzyme activities in the digestive gland of the hybrid abalone. These findings will not only aid the development of multidiet feeding strategies and the use of cost-effective practical feed at different water temperatures throughout the culture period of the hybrid abalone in China but also provide a basis nutrition data for the references to further optimize formulated feed for the hybrid abalone.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no potential conflicts or competing of interest.

Authors' Contributions

Yao-Bin Ma and Wei-Guang Zou contributed equally to this work.

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References

[1] FAO, *The State of World Fsheries and Aquaculture 2020: Sustainability in Action*, Italy, Rome, 2020.

[2] Yearbook, CFS, *China Fishery Statistical Yearbook, 2021. Fishery bureau, ministry of agriculture*, China Agriculture Press, Beijing, 2021.

[3] X. M. Guo, S. E. Ford, and F. S. Zhang, "Molluscan aquaculture in China," *Journal of Shellfish Research*, vol. 18, pp. 19–31, 1999.

[4] L. J. Xu and F. X. Wu, *China Statistical Yearbook 2019*, China Agriculture Press, Beijing, 2019.

[5] S. Liang, X. Luo, W. You, L. Luo, and C. Ke, "The role of hybridization in improving the immune response and thermal tolerance of abalone," *Fish & Shellfish Immunology*, vol. 39, no. 1, pp. 69–77, 2014.

[6] L. Venter, T. L. Du, A. Vosloo, P. Rensburg, and J. Z. Lindeque, "Abalone growth and associated aspects: now from a metabolic perspective," *Reviews in Aquaculture*, vol. 10, no. 2, pp. 451–473, 2018.

[7] W. You, Q. Guo, F. Fan, P. Ren, X. Luo, and C. Ke, "Experimental hybridization and genetic identification of Pacific abalone *Haliotis discus hannai* and green abalone *H. fulgens*," *Aquaculture*, vol. 448, pp. 243–249, 2015.

[8] M. Abdel-Tawwab, "Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, oreochromis niloticus (L.)," *International Aquatic Research*, vol. 4, no. 1, pp. 3–13, 2012.

[9] M. S. Bansemer, D. Stone, T. D'Antignana, P. Skordas, L. Kuerschner, and K. L. Currie, "Optimizing feeding strategies for yellowtail jack at winter water temperatures," *North American Journal of Aquaculture*, vol. 80, no. 2, pp. 128–140, 2018.

[10] L.-S. Cai, L. Wang, K. Song, K.-L. Lu, C.-X. Zhang, and S. Rahimnejad, "Evaluation of protein requirement of spotted seabass (*Lateolabrax maculatus*) under two temperatures, and the liver transcriptome response to thermal stress," *Aquaculture*, vol. 516, 2020.

[11] F. Gauquelin, G. Cuzon, G. Gaxiola et al., "Effect of dietary protein level on growth and energy utilization by *Litopenaeus stylirostris* under laboratory conditions," *Aquaculture*, vol. 271, no. 1-4, pp. 439–448, 2007.

[12] S. Refstie, T. Storebakken, and A. J. Roem, "Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens," *Aquaculture*, vol. 162, no. 3-4, pp. 301–312, 1998.

[13] S. L. Ma, X. X. Li, L. Sun et al., "Effects of dietary protein levels on growth performance, serum indexes, PI3K/AKT/mTOR/S6K signalling and intestinal microbiota of abalone Haliotis discus hannai," *Aquaculture Nutrition*, vol. 27, no. 4, pp. 941–952, 2021.

[14] K. Mai, J. P. Mercer, and J. Donlon, "Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth," *Aquaculture*, vol. 136, no. 1-2, pp. 165–180, 1995.

[15] P. J. Britz, "Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae* , fed fishmeal-based semi-purified diets," *Aquaculture*, vol. 140, no. 1-2, pp. 55–61, 1996.

[16] P. J. Britz and T. Hecht, "Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*," *Aquaculture*, vol. 156, no. 3-4, pp. 195–210, 1997.

[17] J. Sales, P. J. Truter, and P. J. Britz, "Optimum dietary crude protein level for growth in South African abalone (Haliotis midaeL.)," *Aquaculture Nutrition*, vol. 9, no. 2, pp. 85–89, 2003.

[18] T. A. Coote, P. W. Hone, R. J. Van Barneveld, and G. B. Maguire, "Optimal protein level in a semipurified diet for juvenile greenlip abaloneHaliotis laevigata," *Aquaculture Nutrition*, vol. 6, no. 4, pp. 213–220, 2000.

[19] D. A. J. Stone, J. O. Harris, H. R. Wang, G. J. Mercer, E. N. Schaefer, and M. S. Bansemer, "Dietary protein level and water temperature interactions for greenlip abalone Haliotis laevigata," *Journal of Shellfish Research*, vol. 32, no. 1, pp. 119–130, 2013.

[20] G. A. Dunstan, "A simple model for the determination of the relative utilization efficiency of protein by blacklip abalone (Haliotis rubra leach)," *Aquaculture Nutrition*, vol. 16, no. 1, pp. 1–12, 2010.

[21] M. N. Bautista-Teruel and O. M. Millamena, "Diet development and evaluation for juvenile abalone, *Haliotis asinina* : protein/energy levels," *Aquaculture*, vol. 178, no. 1-2, pp. 117–126, 1999.

[22] C. H. Tung and A. C. Alfaro, "Effect of dietary protein and temperature on the growth and health of juvenile New Zealand black-footed abalone (Haliotis iris)," *Aquaculture Research*, vol. 42, no. 3, pp. 366–385, 2011.

[23] J. Montañovargas, M. T. Viana, L. R. D'Abramo, A. Shimada, and C. Vasquez-Pelaez, "Growth and energy utilization of juvenile pink abalone Haliotis corrugata fed diets containing different levels of protein and two starch: lipid ratios," *Journal of Shellfish Research*, vol. 24, no. 4, pp. 1179–1185, 2005.

[24] M. R. Catacutan and R. M. Coloso, "Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*," *Aquaculture*, vol. 131, no. 1-2, pp. 125–133, 1995.

[25] M. A. Naylor, H. Kaiser, and C. L. W. Jones, "The effect of dietary protein level on total ammonia nitrogen and free ammonia nitrogen concentrations in a serial-use raceway used to farm South African abalone, Haliotis midaeLinnaeus, 1758," *Journal of Shellfish Research*, vol. 30, no. 2, pp. 337–341, 2011.

[26] R. K. Singh, A. S. Desai, S. L. Chavan, and P. A. Khandagale, "Effect of water temperature on dietary protein requirement, growth and body composition of Asian catfish, *Clarias batrachus* fry," *Journal of Thermal Biology*, vol. 34, no. 1, pp. 8–13, 2009.

[27] J. H. Tidwell, C. D. Webster, and S. D. Coyle, "Effects of dietary protein level on second year growth and water quality for largemouth bass (*Micropterus salmoides*) raised in ponds," *Aquaculture*, vol. 145, no. 1-4, pp. 213–223, 1996.

[28] J. S. Huang, Z. X. Guo, J. D. Zhang et al., "Effects of hypoxia-reoxygenation conditions on serum chemistry indicators and gill and liver tissues of cobia (*Rachycentron canadum*)," *Aquaculture Reports*, vol. 20, article 100692, 2021.

[29] S. L. Ma, Y. L. Guo, L. Sun et al., "Over high or low dietary protein levels depressed the growth, TOR signaling, apoptosis, immune and anti-stress of abalone Haliotis discus hannai," *Fish & Shellfish Immunology*, vol. 106, pp. 241–251, 2020.

[30] N. N. Zeng, M. Jiang, H. Wen et al., "Effects of water temperatures and dietary protein levels on growth, body composition and blood biochemistry of juvenile GIFT tilapia (Oreochromis niloticus)," *Aquaculture Nutrition*, vol. 27, pp. 240–251, 2021.

[31] S. Jiang, F. L. Zhou, Q. B. Yang, J. H. Huang, L. S. Yang, and S. G. Jiang, "Impact of temperature stress on oxygen and energy metabolism in the hepatopancreas of the black Tiger shrimp, Penaeus monodon (Crustacea: Decapoda: Penaeidae)," *Pakistan Journal of Zoology*, vol. 51, pp. 141–148, 2018.

[32] D. L. Wu, Z. Q. Liu, Y. H. Huang et al., "Effects of cold acclimation on the survival, feeding rate, and non-specific immune responses of the freshwater red claw crayfish (Cherax quadricarinatus)," *Aquaculture International*, vol. 26, no. 2, pp. 557–567, 2018.

[33] F. Yu, W. Peng, B. Tang et al., "A genome-wide association study of heat tolerance in Pacific abalone based on genome resequencing," *Aquaculture*, vol. 536, article 736436, 2021.

[34] X. Yan, J. Yang, X. Dong et al., "The protein requirement of grouper Epinephelus coioides at grow-out stage," *Aquaculture Nutrition*, vol. 26, no. 5, pp. 1555–1567, 2020.

[35] AOAC, *Official Methods of Analysis of the Association of Official Analytical Chemists*, Association of Official Analytical Chemists, Arlington, VA, USA, 16th ed. edition, 1995.

[36] J. Yin, H. Lu, Q. Xie, J. Ding, and N. Li, "A study on rapid colorimetric determination of water soluble total sugar, reducing sugar and starch in tobacco with 3,5-dinitrosalicylic acid," *J. Yunnan Agr. U.*, vol. 6, pp. 829–833, 2007.

[37] A. S. Keston and R. Brandt, "The fluorometric analysis of ultramicro quantities of hydrogen peroxide," *Analytical Biochemistry*, vol. 11, no. 1, pp. 1–5, 1965.

[38] A. O. Teles, A. Couto, P. Enes, and H. Peres, "Dietary protein requirements of fish – a meta-analysis," *Reviews in Aquaculture*, vol. 12, article raq.12391, pp. 1445–1477, 2019.

[39] M. N. Amin, R. K. Barnes, and L. R. Adams, "Effect of temperature and varying level of carbohydrate and lipid on growth, feed efficiency and nutrient digestibility of brook trout, *Salvelinus fontinalis* (Mitchill, 1814)," *Animal Feed Science and Technology*, vol. 193, pp. 111–123, 2014.

[40] T. Akutsu and N. Okamoto, "Influência de alterações de temperatura na agregação de trombócitos em truta arco-íris," *Brazilian Journal of Veterinary Research and Animal Science*, vol. 43, no. supl, pp. 23–27, 2006.

[41] E. N. Schaefer, J. O. Harris, G. S. Howarth, M. S. Bansemer, and D. A. J. Stone, "Comparative histological changes in the greenlip abaloneHaliotis laevigata gastrointestinal tract in response to water temperature, different dietary protein levels, and animal age," *Journal of Shellfish Research*, vol. 32, no. 1, pp. 131–141, 2013.

[42] Abdel-Fattah & El-Sayed, "Is dietary taurine supplementation beneficial for farmed fish and shrimp? A comprehensive review," *Reviews in Aquaculture*, vol. 6, no. 4, pp. 241–255, 2014.

[43] F. Coutinho, H. Peres, I. Guerreiro, P. Pousão-Ferreira, and A. Oliva-Teles, "Dietary protein requirement of sharpsnout sea bream (Diplodus puntazzo , Cetti 1777) juveniles," *Aquaculture*, vol. 356, pp. 391–397, 2012.

[44] J. Wang, Y. Jiang, X. Li et al., "Dietary protein requirement of juvenile red spotted grouper (*Epinephelus akaara*)," *Aquaculture*, vol. 450, pp. 289–294, 2016.

[45] Y. Gao, S. Lu, M. Wu, W. Yao, and X. Wu, "Effects of dietary protein levels on growth, feed utilization and expression of growth related genes of juvenile giant grouper (*Epinephelus lanceolatus*)," *Aquaculture*, vol. 504, pp. 369–374, 2019.

[46] M. Meer, M. Machiels, and M. Verdegem, "The effect of dietary protein level on growth, protein utilization and body composition ofColossoma macropomum(Cuvier)," *Aquaculture Research*, vol. 26, no. 12, pp. 901–909, 1995.

[47] P. Rema, L. Conceição, F. Evers, M. Castro-Cunha, and M. Dinis, "Optimal dietary protein levels in juvenile Senegalese sole (Solea senegalensis)," *Aquaculture Nutrition*, vol. 14, no. 3, pp. 263–269, 2008.

[48] L. O. Kim and S. M. Lee, "Effects of the dietary protein and lipid levels on growth and body composition of bagrid catfish, *Pseudobagrus fulvidraco*," *Aquaculture*, vol. 243, no. 1-4, pp. 323–329, 2005.

[49] S. S. Giri, S. K. Sahoo, A. K. Sahu, and P. K. Meher, "Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid *Clarias* catfish (*Clarias batrachus* × *Clarias gariepinus*)," *Animal Feed Science and Technology*, vol. 104, no. 1-4, pp. 169–178, 2003.

[50] J. M. Vergara, H. Fernandez-Palacios, L. Robaina, K. Jauncey, L. H. M. De, and M. Izquierdo, "The effects of varying dietary protein level on the growth, feed efficiency, protein utilization and body composition of gilthead sea bream fry," *Fisheries Science*, vol. 62, no. 4, pp. 620–623, 1996.

[51] X. Yan, J. Yang, X. Dong et al., "Optimum protein requirement of juvenile orange-spotted grouper (*Epinephelus coioides*)," *Scientific Reports*, vol. 11, no. 1, p. 6230, 2021.

[52] E. L. Guy, M. H. Li, and P. J. Allen, "Effects of dietary protein levels on growth and body composition of juvenile (age-1) Black Buffalo *Ictiobus niger*," *Aquaculture*, vol. 492, pp. 67–72, 2018.

[53] K. D. Shearer, "Factors affecting the proximate composition of cultured fishes with emphasis on salmonids," *Aquaculture*, vol. 119, no. 1, pp. 63–88, 1994.

[54] S. Fatma and I. Ahmed, "Effect of water temperature on protein requirement of Heteropneustes fossilis (Bloch) fry as determined by nutrient deposition, hemato-biochemical parameters and stress resistance response," *Fisheries and Aquatic Sciences.*, vol. 23, no. 1, pp. 1–14, 2020.

[55] Z. Sun, S. Xia, S. Feng et al., "Effects of water temperature on survival, growth, digestive enzyme activities, and body composition of the leopard coral grouper Plectropomus leopardus," *Fisheries Science*, vol. 81, pp. 107–112, 2015.

[56] Y. Cui and R. J. Wootton, "Effects of ration, temperature and body size on the body composition, energy content and condition of the minnow, Phoxinus phoxinus (L.)," *Journal of Fish Biology*, vol. 32, no. 5, pp. 749–764, 1988.

[57] R. E. Olsen and E. Ringo, "The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, Salvelinus alpinus L," *Aquaculture Research.*, vol. 29, no. 10, pp. 695–701, 1998.

[58] T. T. Zhu, M. Jin, Y. M. Hou et al., "Effects of different dietary lipid sources on growth performance, antioxidant enzyme activities and biochemical composition of juvenile swimming crab, Portunus trituberculatus," *Aquaculture Nutrition.*, vol. 25, no. 6, pp. 1440–1450, 2019.

[59] C. D. Berdanier, "Metabolism: carbohydrate, lipid and protein," *Encyclopedia of Gerontology.*, vol. 2, pp. 167–175, 2007.

[60] D. E. Kelley, "Sugars and starch in the nutritional management of diabetes mellitus," *American Journal of Clinical Nutrition*, vol. 78, no. 4, pp. 858S–864S, 2003.

[61] E. John and R. David, *Geriatric Nutrition*, CRC Press, Florida, USA, 2007.

[62] I. Bairati, L. Roy, and F. Meyer, "Effects of a fish oil supplement on blood pressure and serum lipids in patients treated for coronary artery disease," *The Canadian Journal of Cardiology.*, vol. 8, no. 1, pp. 41–46, 1992.

[63] M. E. Burt, T. P. Stein, J. G. Schwade, and M. F. Brennan, "Whole-body protein metabolism in cancer-bearing patients. Effect of total parenteral nutrition and associated serum insulin response," *Cancer*, vol. 53, no. 6, pp. 1246–1252, 1984.

[64] M. F. Nazarudin, M. Aliyu-Paiko, and M. N. Shamsudin, "Serum cortisol concentrations change in tiger grouper, Epinephelus fuscoguttatus in response to water temperature and salinity stress," *Iranian Journal of Fisheries Sciences*, vol. 15, pp. 1511–1525, 2016.

[65] F. G. Nordlie, "Environmental influences on regulation of blood plasma/serum components in teleost fishes: a review," *Reviews in Fish Biology and Fisheries*, vol. 19, no. 4, pp. 481–564, 2009.

[66] L. T. Enevoldsen, I. Heiner, A. L. Devries, and J. F. Steffensen, "Does fish from the Disko Bay area of Greenland possess antifreeze proteins during the summer?," *Polar Biology*, vol. 26, no. 6, pp. 365–370, 2003.

[67] J. F. Huang, X. U. Qi-You, C. A. Wang, Z. G. Zhao, and L. Luo, "Effects of temperature and dietary protein content on the blood biochemical parameters in juvenile Songpu mirror carp Cyprinus specularis Songpu," *Journal of Dalian Ocean University.*, vol. 26, pp. 41–46, 2013.

[68] A. Talukdar, A. D. Deo, N. P. Sahu et al., "Effects of dietary protein on growth performance, nutrient utilization, digestive enzymes and physiological status of grey mullet, Mugil cephalus L. fingerlings reared in inland saline water," *Aquaculture Nutrition*, vol. 26, no. 3, pp. 921–935, 2020.

[69] S. Zhu, X. Long, G. M. Turchini, D. Deng, Y. Cheng, and X. Wu, "Towards defining optimal dietary protein levels for male and female sub-adult Chinese mitten crab, *Eriocheir sinensis* reared in earthen ponds: performances, nutrient composition and metabolism, antioxidant capacity and immunity," *Aquaculture*, vol. 536, article 736442, 2021.

[70] C. Xu, W. B. Liu, D. D. Zhang, X. F. Cao, H. J. Shi, and X. F. Li, "Interactions between dietary carbohydrate and metformin: implications on energy sensing, insulin signaling pathway, glycolipid metabolism and glucose tolerance in blunt snout bream *Megalobrama amblycephala*," *Aquaculture*, vol. 483, pp. 183–195, 2018.

[71] J. Guo, Y. Fu, Z. Wu et al., "Effects of dietary carbohydrate levels on growth performance, body composition, glucose/lipid metabolism and insulin signaling pathway in abalone *Haliotis discus hannai*," *Aquaculture*, vol. 557, article 738284, 2022.

[72] J. G. Dorea, "Persistent, bioaccumulative and toxic substances in fish: human health considerations," *Science of the Total Environment*, vol. 400, no. 1-3, pp. 93–114, 2008.

[73] R. Sá, P. Pouso-Ferreira, and A. Oliva-Teles, "Growth performance and metabolic utilization of diets with different protein:carbohydrate ratios by white sea bream (Diplodus sargus, L.) juveniles," *Aquaculture Research*, vol. 38, no. 1, pp. 100–105, 2007.

[74] S. S. Vutukuru, N. A. Prabhath, M. Raghavender, and A. Yerramilli, "Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, Labeo rohita," *International Journal of Environmental Research and Public Health*, vol. 4, no. 3, pp. 224–227, 2007.

[75] T. Roszer, "The invertebrate midintestinal gland ("hepatopancreas") is an evolutionary forerunner in the integration of immunity and metabolism," *Cell and Tissue Research*, vol. 358, no. 3, pp. 685–695, 2014.

[76] Y. Fu, J. Guo, Z. Wu et al., "Effects of dietary chromium yeast levels on growth performance, anti-oxidative capacity, immune response and flesh quality of abalone *Haliotis discus hannai*," *Aquaculture*, vol. 557, article 738291, 2022.

[77] L. Cheng, Q. Qin, J. Li et al., "Effect of dietary with different protein levels on the growth, digestive enzyme activities and body composition of the juvenile Spinibarbus hollandi Oshima," *Feed Industry.*, vol. 39, pp. 34–39, 2018.

[78] F. Wu, H. Wen, M. Jiang et al., "Effects of different dietary carbohydrate levels on growth performance and blood biochemical parameters of juvenile GIFT tilapia (Oreochromis niloticus)," *Journal of Northwest A & F University.*, vol. 40, pp. 9–14, 2012.

[79] H. Tsutsui, S. Kinugawa, and S. Matsushima, "Oxidative stress and heart failure," *American Journal of Physiology. Heart and Circulatory Physiology*, vol. 301, no. 6, pp. H2181–H2190, 2011.

[80] U. Pantke, T. Volk, M. Schmutzler, W. J. Kox, N. Sitte, and T. Grune, "Oxidized proteins as a marker of oxidative stress

during coronary heart surgery," *Free Radical Biology & Medicine*, vol. 27, no. 9-10, pp. 1080–1086, 1999.

[81] D. Vosloo, L. van Rensburg, and A. Vosloo, "Oxidative stress in abalone: the role of temperature, oxygen and L-proline supplementation," *Aquaculture*, vol. 416, pp. 265–271, 2013.

[82] B. Lv, B. Liu, Q. Zhou et al., "Effects of different temperatures and protein levels on growth performance, physiological response and expression of immune-related genes of juvenile oriental river prawn (Macrobrachium nipponense)," *Aquaculture*, vol. 536, article 736435, 2021.

[83] H. Zhang, F. Chen, Z. Liang, Y. Wu, and Y. Pu, "Analysis of miRNAs and their target genes associated with mucosal damage caused by transport stress in the mallard duck intestine," *PLoS One*, vol. 15, no. 8, article e0237699, 2020.

[84] S. Venkatesan and K. Pugazhendy, "Chelating properties of spirulina against the atrazine toxicity on the antioxidant enzymes activities in the fresh water fish cyprinus carpio (Linn)," *International Journal of Recent Scientific Research.*, vol. 3, pp. 181–186, 2011.