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Effects of Water Velocity on Growth, Physiology and Intestinal Structure of Coral Trout (*Plectropomus leopardus*)

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Abstract: This study aimed to investigate the effects of different water velocities on the growth performance, blood physiology, and digestive capacity of coral trout (*Plectropomus leopardus*) in a Recirculating aquaculture system (RAS). One hundred and twenty healthy, uniformly sized coral trout (body mass (92.01 ± 8.04) g; body length (15.40 ± 0.65) cm) were randomly assigned to three flow velocity groups (1 bl/s, 2 bl/s, and 2.5 bl/s) and one control group (0 bl/s). The results show that the weight gain rate (WGR) and specific growth rate (SGR) of coral trout in the 2.5 bl/s water flow velocity group were significantly lower than those in the control group and 1 bl/s water flow velocity group ($p < 0.05$), while their feed coefficient (FC) values were significantly higher than those of the control group and 1 bl/s water flow velocity group ($p < 0.05$). The blood glucose (GLU) concentration of coral trout in the 2 bl/s water flow velocity group and the 2.5 bl/s water flow velocity group significantly decreased compared to those in the control group ($p < 0.05$), while the lactic acid (LD) concentration increased. As the cortisol (COR) concentration and lipase (LPS) enzyme activity of coral trout did not significantly change ($p > 0.05$), the α- AMS enzyme activity significantly decreased ($p < 0.05$). Under 2.5 bl/s water flow velocity, the intestinal structure of coral trout changed, and the number of goblet cells decreased. High-water flow velocities affect the physiological homeostasis and intestinal digestion of coral trout, resulting in a decrease in their growth performance, indicating that coral trout is more sensitive to high-water flow velocities. In actual RAS aquaculture, the flow rate should be controlled within 1 bl/s.

Keywords: *Plectropomus leopardus*; water flow velocity; growth performance; blood biochemistry; intestinal digestibility

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1. Introduction

Plectropomus leopardus, commonly known as coral trout, is one of the most important species cultured in China, with such advantages as delicious meat, bright color, and rich nutritional value [1]. The limitations of the marine fishing industry and its high commercial value have promoted the development of the artificial breeding of coral trout [2]. China is a late adopter in the captive breeding of coral trout, and the production in this country is far from sufficient to meet the growing demand. There are currently two mainstream farming methods for coral trout, flowing water culturing, and cage culturing [3]. Flowing water culturing entails a serious waste of water and does not meet today's interests in water and energy conservation. Cage culturing may lead to waves and typhoons, which can cause some pollution to offshore waters. Recirculating aquaculture system (RAS) is developed for intensive aquaculture, which can save water resources and land resources, protect the environment, and help regulate the aquaculture water quality to provide the best aquaculture environment for fish [4]. The fish cultured under RAS have significant advantages in terms of food quality and safety. Coral trout is a warm-water reef fish that prefers to live near coral reefs with low flow velocities [5]. However, the influence of water flow velocity is often ignored in the artificial cultivation of coral trout.

As an environmental factor, water velocity also significantly affects the growth performance, morphological characteristics, and physiological changes of fish [6]. Bl/s is the body length per second. The tolerable water flow velocity of fish is related to their body length [7]. A previous study has reported that high-velocity and high-intensity continuous exercise training (2.0 bl/s) will reduce the growth rate and food conversion rate of juvenile tinfoil barbs (*Barbodes schwanenfeldi*) (15.12 ± 1.35 cm) [8]. Conversely, largemouth bass (*Micropterus salmoides*) (4.50 ± 0.36 cm) can show improved growth performance, digestion, and immunity at a high-water flow velocity (4.0 bl/s) [9]. *Salmo salar* (19.26 ± 0.08 cm) can obtain the best growth performance at 1 bl/s water flow velocity [7]. *Scophthalmus maximus* juvenile (161.9 ± 8 g) shows a reduced feed coefficient and improved specific growth rate at medium water flow velocity [10]. However, there are few reports on the appropriate water flow velocity for breeding coral trout. To optimize the aquaculture facilities and improve the aquaculture environment, it is essential to determine the appropriate water flow velocity for this species.

In this study, the growth performance, blood physiological changes, intestinal digestive enzyme activity, and digestive system structure of fish at different water flow velocities were used as indicators to explore the appropriate water flow velocities for coral trout in RAS. The results from the present study will provide a theoretical basis for the circulation of water culturing of coral trout and promote the healthy and sustainable development of aquaculture.

2. Materials and Methods

2.1. Experimental Fish and Design

A batch of juvenile coral trout was obtained from Delin Chengxin Aquaculture Co., Ltd. (Lingshui, China) with an initial body weight of 92.01 ± 8.04 g and a body length of 15.40 ± 0.65 cm. The culture experiments were carried out in a recirculating water culture tank at the pilot plant of the Institute of Fisheries Machinery and Instruments of the Chinese Academy of Fisheries Sciences (Figure 1). The system consists of a culture tank, a push-flow water pump, a wet and dry separation filter, a moving bed, a UV sterilization device, and a reservoir. The mobile bed has an oxygenating and aeration device to ensure the presence of sufficient dissolved oxygen in the experimental setup. Each tank is equipped with a push-flow water pump, and the intended water flow velocity is provided by a pump (Pond Spring, ABB-200), pushing the water at a speed of 0.5–7.5 bl/s (10–120 cm/s) in the tank.

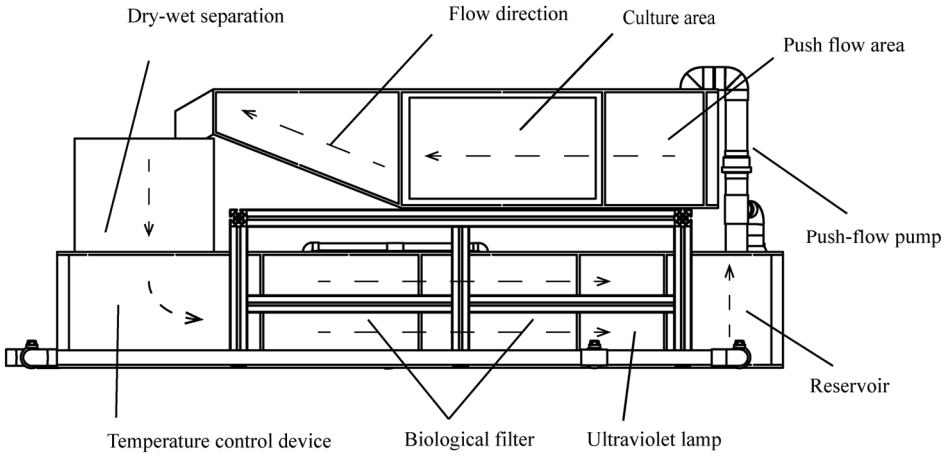

Figure 1. Circulating water culture tank.

The experiment was divided into three water flow velocity groups, with water flow velocities of 1 bl/s, 2 bl/s, and 2.5 bl/s. In addition, a control group was set up with a water flow velocity of 0 bl/s. Each group contained 3 tanks, and the experimental fish were evenly allocated to each tank. There were 10 fish in each tank and a total of 120 fish in the experiment. After one week of acclimatization, the experiment started and then lasted for

42 d. During the experimental period, the fish were fed at 2% of their body mass daily. The fish were fed three times daily at 8:00, 13:00, and 18:00, and the amount of food and residual bait was recorded. Body weight and length measurements were conducted on the 7th, 14th, 21st, 28th, 35th, and 42nd days of the experimental period to adjust the water flow velocity and feeding rate. A total of 6 fish (2 fish per tank) were collected from the control group and the different water flow velocity groups. They were weighed with water and put back into the original tank after weighing. We ensured that the feeding amount was always maintained at 2% body weight and that the water flow velocity was maintained at 1 bl/s, 2 bl/s, and 2.5 bl/s. The water temperature was maintained at 26.5 ± 1.5 °C, salinity 22–24‰, pH 8.07–8.31, dissolved oxygen 8.08 ± 0.26 mg/L, ammonia nitrogen 0.07 ± 0.05 mg/L, and nitrite 0.18 ± 0.12 mg/L.

2.2. Sample Collection and Processing

2.2.1. Sample Collection

On day 42, the experimental fish were sampled for blood samples, and intestinal and liver tissue. Before sampling, the experimental fish were starved for 24 h, and a total of 6 fish were collected from each experimental group (2 per tank). They were put into MS-222 water with a concentration of 120 mg/L for rapid anesthesia, and their body length was measured and weighed. First, blood was collected from the caudal vertebral vein of the experimental fish, and then the fish's body was dissected. The visceral mass was taken out and weighed along with the liver. The intestines were separated from the visceral mass, and the foregut, midgut, and hindgut were collected and transferred to a 10% concentration of formalin for preservation, which was used for the observation of intestinal tissue sections. The rest of the intestines were placed directly into a cryopreservation tube and stored in liquid nitrogen.

2.2.2. Sample Handling

After all the samples were collected, the blood samples were centrifuged at 3500 rpm for 10 min at 4 °C using a cryogenic freezing centrifuge, and the upper serum layer was packed into lyophilized tubes and stored in a −80 °C refrigerator. The intestinal tissues were stored in a −80 °C refrigerator.

After thawing, the intestinal tissues were accurately weighed and added to 9 times the volume of saline at a ratio of mass (g): volume (mL) = 1:9, which was then homogenized at 4 °C using a freezing homogenizer (MYD-48) to produce a 10-fold dilution of homogenate. The supernatant was then centrifuged at 2500 r/min for 10 min at 4 °C, after which it was used to determine the activity of intestinal digestive enzymes.

2.3. Index Measurement

2.3.1. Growth Performance Parameters' Measurements

Feed coefficient (FC), weight gain rate (WGR, %), specific growth rate (SGR, %), condition factor (CF, %), hepatosomatic index (HSI, %), and viscerosomatic index (VSI, %) were calculated as follows:

$$FC = W_f/(W_t - W_0), \quad (1)$$

$$WGR = (W_t - W_0)/W_0 \times 100\%, \quad (2)$$

$$SGR = (\ln W_t - \ln W_0)/t \times 100\%, \quad (3)$$

$$CF = W_t/L^3 \times 100\%, \quad (4)$$

$$HSI = W_l/W_t \times 100\%, \quad (5)$$

$$VSI = W_v/W_t \times 100\%. \quad (6)$$

W_f is the total feeding of the fish on day t at the time of measurement, W_t is the mass of the fish measured at the time of the final sampling (g), W_0 is the mass of the fish measured at the time of the initial sampling (g), t is the number of days of breeding (d), L is the body length of the fish at the time of measurement (cm), W_l is the mass of the fish's liver at the time of sampling (g), and W_v is the mass of the fish's visceral mass at the time of sampling (g).

2.3.2. Blood Biochemical Parameters Measurements

Blood glucose (GLU) concentrations were measured via the glucose oxidase method, blood lactic acid (LD) concentrations were measured via the colorimetric method, and blood cortisol (COR) was measured via the double antibody sandwich method with an enzyme-linked immunoassay kit. All samples were tested according to the operating instructions of the kit (Jiancheng Institute of Biological Engineering, Nanjing, China).

2.3.3. Intestinal Digestive Enzyme Activity Measurements

Protein concentrations in intestinal tissues were determined by the Komas Brilliant Blue method. Intestinal α-amylase (α-AMS) and lipase (LPS) activities were determined via the enzyme starch colorimetric method and enzyme colorimetric method, respectively. All samples were assayed according to the operating instructions of the kit (Jiancheng Bioengineering Institute, Nanjing, China).

2.3.4. Fixation and Observation of Intestinal Section

The foregut, midgut, and hindgut of the experimental fish were fixed in 10% formalin for 24 h, dehydrated in 70%, 80%, 90, 95%, and anhydrous alcohol in that order, transplanted into xylene, embedded in paraffin, sectioned (5 μm) with a microtome (Leica RM2235, Wetzlar, Germany), then stained with hematoxylin and eosin, and sealed with neutral gum. A microscope (Leica DM1000, Wetzlar, Germany) was used to observe the intestinal tissues (magnification of intestinal tissues was 10). Image J software was used to measure the intestinal muscular thickness (MS), intestinal villus length (VL), and villus thickness (VT).

2.4. Statistical Analysis

Excel 2019 software was used for data processing. All statistical analyses were performed with SPSS 26.0 software. All figures were drawn using the Prism GraphPad 9 software. The data obtained in the text were subjected to one-way ANOVA followed by Duncan's multiple comparison analysis to determine if there were any significant differences ($p < 0.05$), and the experimental results are expressed as mean ± standard deviation (mean ± SD). Assumptions of normality, equality of variances, and outliers were confirmed prior to statistical analysis.

3. Results

3.1. Growth Performance

The statistical results show significant differences in fish growth performance among the experimental groups ($p < 0.05$, Table 1). The WGR and SGR of the coral trout in the 2.5 bl/s water flow velocity group were significantly lower than those in the other groups ($p < 0.05$), showing also significantly higher FC values than the control and 1 bl/s water flow velocity groups, the lowest feed conversion rate, and the lowest weight gain. The VSI of fish in the 2 bl/s water flow velocity group was the lowest, significantly lower than that of the control group ($p < 0.05$) and not significantly among the water flow velocity groups ($p > 0.05$). There was no significant difference ($p > 0.05$) among the water flow velocity groups as regards the CF and HSI of coral trout.

Table 1. Effect of water flow velocity on growth performance of coral trout.

Item	Control	1 bl/s Water Flow Velocity	2 bl/s Water Flow Velocity	2.5 bl/s Water Flow Velocity
FC	1.17 ± 0.14 ^{b}	1.14 ± 0.12 ^{b}	1.36 ± 0.21 ^{ab}	1.48 ± 0.23 ^{a}
WGR (%)	47.54 ± 5.63 ^{a}	47.73 ± 5.55 ^{a}	40.44 ± 5.63 ^{ab}	37.92 ± 5.57 ^{b}
SGR (%)	0.93 ± 0.09 ^{a}	0.93 ± 0.09 ^{a}	0.81 ± 0.10 ^{ab}	0.76 ± 0.10 ^{b}
CF (%)	2.53 ± 0.20	2.68 ± 0.05	2.63 ± 0.10	2.51 ± 0.08
HIS (%)	1.16 ± 0.16	1.22 ± 0.07	1.33 ± 0.18	1.24 ± 0.01
VSI (%)	5.06 ± 0.48 ^{a}	4.70 ± 0.42 ^{ab}	4.25 ± 0.13 ^{b}	4.40 ± 0.41 ^{ab}

The values are the mean ± standard deviation of four replications (n = 6). Values with different letters imply a significant difference among the groups ($p < 0.05$), and no letters means no significant difference ($p > 0.05$).

3.2. Blood Biochemistry

Water flow velocity significantly affected the blood biochemistry of the fish (Figure 2). The GLU concentrations of coral trout were significantly higher in the control and 1 bl/s groups than in the 2 bl/s and 2.5 bl/s water flow velocity groups ($p < 0.05$), and LD concentrations were lowest in the 1 bl/s water flow velocity group, which were significantly lower than in the 2.5 bl/s group ($p < 0.05$), with no significant difference from the control and 2 bl/s groups ($p > 0.05$). There was no significant difference in blood COR concentration of coral trout among the water flow velocity groups ($p > 0.05$) and no significant effect of water velocity on coral trout stress levels ($p > 0.05$).

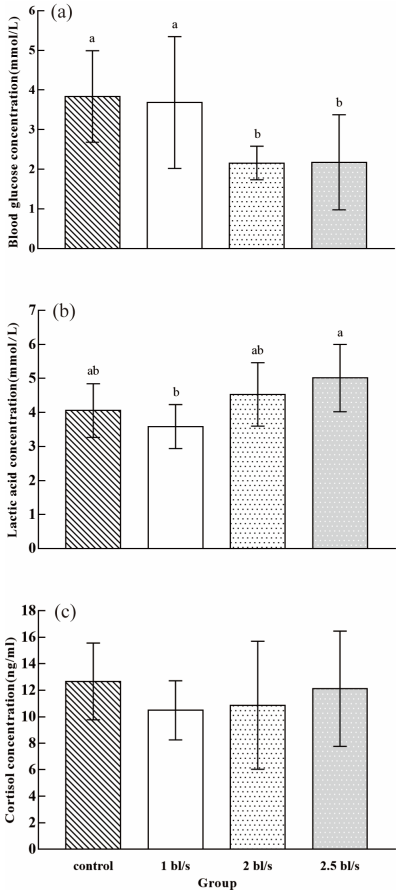

Figure 2. Effect of water flow velocity on blood biochemistry of fish (*Plectropomus leopardus*), (n = 6). (**a**) Blood glucose concentration; (**b**) lactic acid concentration; (**c**) cortisol concentration. Different letters indicate significant differences among groups ($p < 0.05$). No letter means no significant difference among groups ($p > 0.05$).

3.3. Intestinal Digestive Enzyme Activity

Figure 3a shows that the intestinal LPS activity of coral trout was not affected by water flow velocity ($p > 0.05$) and was maintained at normal levels in all groups. Intestinal α-AMS activity was significantly ($p < 0.05$) affected by water flow velocity (Figure 3b), with the values in the control and 1 bl/s water flow velocity groups both significantly higher than in the 2 bl/s and 2.5 bl/s water flow velocity groups ($p < 0.05$), with no significant difference among the control and 1 bl/s water flow velocity groups ($p > 0.05$). Water flow velocities above 2 bl/s reduced the digestive capacity of the fish intestine and affected growth and development.

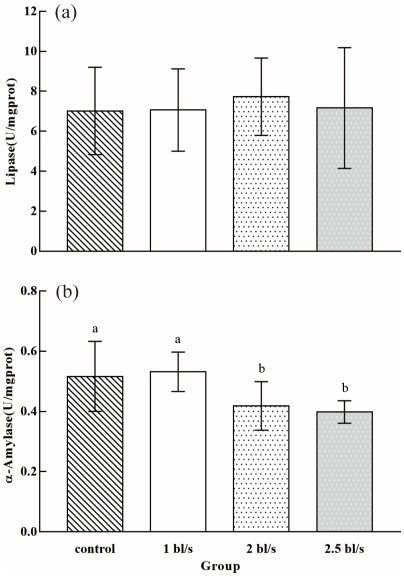

Figure 3. Effect of water flow velocity on the activity of intestinal digestive enzymes in fish (*Plectropomus leopardus*), (n = 6). (**a**) Lipase activity; (**b**) α-amylase activity. Different letters indicate significant differences among groups ($p < 0.05$). No letter means no significant difference among groups ($p > 0.05$).

3.4. Intestinal Structure

3.4.1. Foregut

Table 2 shows that the lengths of foregut VL of coral trout in the different water flow velocity groups were significantly different ($p < 0.05$) and that the 1 bl/s water flow velocity group was significantly shorter than those of the control group and other water flow velocity groups ($p < 0.05$). The value of MS in the 2.5 bl/s water flow velocity group was significantly lower than in the 2 bl/s water flow velocity group ($p < 0.05$). The VT of coral trout in the 2 bl/s velocity group was the highest—significantly higher than in the control and other velocity groups ($p < 0.05$). The villous tissue structure of coral trout in each water flow velocity group was complete, but the number of goblet cells in the medium–high-water flow velocity group (2 bl/s and 2.5 bl/s) was reduced compared with the control group (Figure 4).

Table 2. Effect of water flow velocity on the index of foregut tissue of coral trout.

Item	Control	1 bl/s Water Flow Velocity	2 bl/s Water Flow Velocity	2.5 bl/s Water Flow Velocity
VL (μm)	749.83 ± 21.17 ^{a}	565.72 ± 25.18 ^{b}	832.54 ± 59.57 ^{a}	764.4 ± 64.33 ^{a}
MS (μm)	198.72 ± 7.92a ^{b}	202.85 ± 38.01 ^{ab}	227.49 ± 20.69 ^{a}	190.8 ± 21.5 ^{b}
VT (μm)	60.72 ± 7.28 ^{b}	54.54 ± 7.65 ^{b}	84.31 ± 12.74 ^{a}	68.58 ± 7.12 ^{b}

The values are average ± standard deviation of four replications (n = 6). Values of different letters mean significant difference among the groups ($p < 0.05$), and those with no letters mean no significant difference ($p > 0.05$).

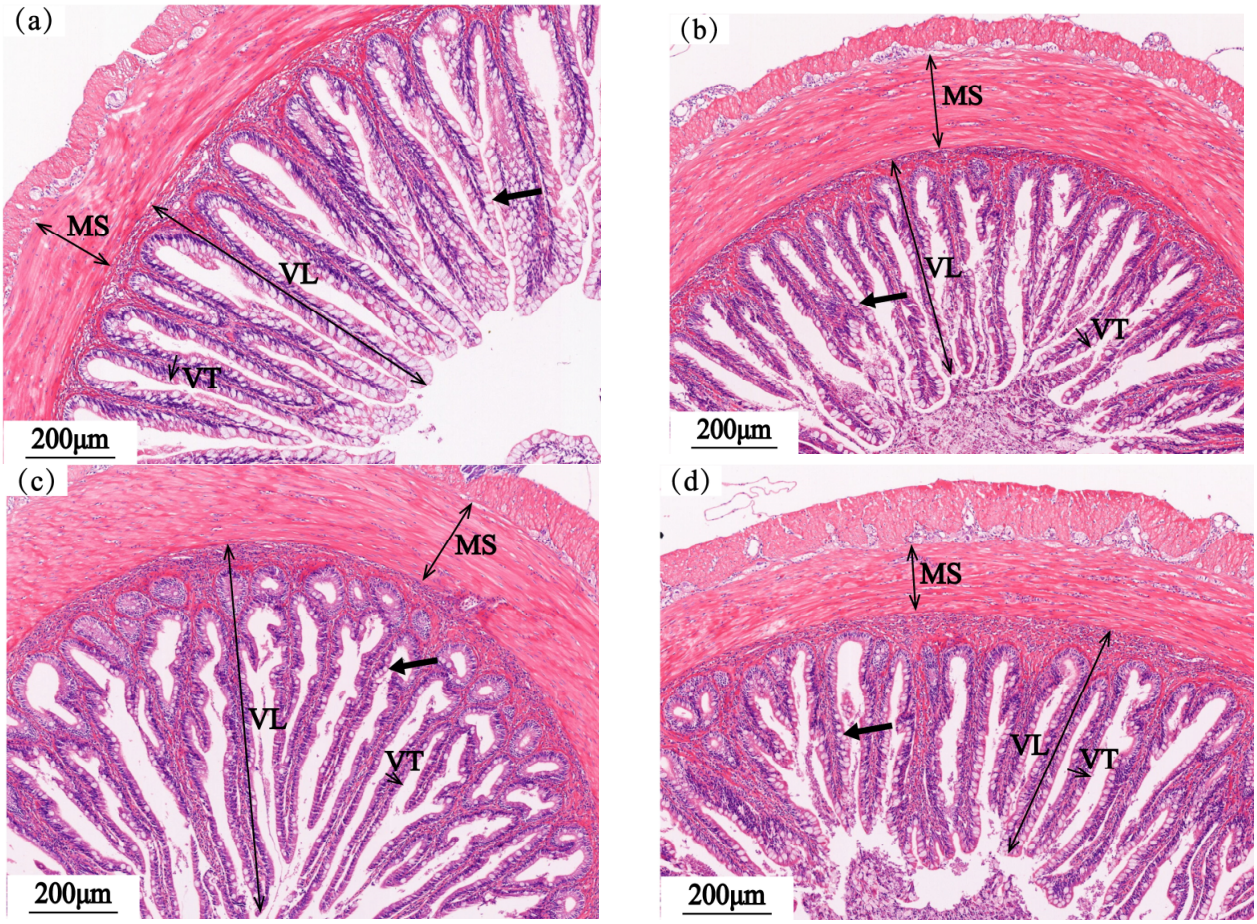

Figure 4. The effect of water flow velocity on the foregut structure of coral trout. (**a**) Hydrostatic control group: intestinal tissue was normal, muscular thickness (MS), villus length (VL), and villus thickness (VT) were uniform, and there were many goblet cells (thick arrows); (**b**) 1 bl/s water flow velocity group: muscular thickness (MS), villus length (VL), and villus thickness (VT) showed no significant changes compared with the control group, while goblet cells (thick arrows) were smaller and their number was relatively reduced; (**c**) 2 bl/s water flow velocity group: the muscle layer was thinner than in the control, while the number of goblet cells (thick arrows) was relatively reduced and their size was smaller; (**d**) 2.5 bl/s water flow velocity group: the muscle layer was relatively thin, while the number of goblet cells (thick arrows) in the contrast photograph was relatively small, and the morphological distribution was uneven.

3.4.2. Midgut

Table 3 shows the index values of the midgut tissues of coral trout in different water flow velocity groups, indicating significant differences among the groups. There was no significant difference in coral trout's VL among the 1 bl/s water flow velocity group and the control group ($p > 0.05$), but it was significantly higher than in other water flow velocity groups ($p < 0.05$). Compared with the control group and other velocity groups, the MS value of coral trout in the 2 bl/s velocity group was the highest ($p < 0.05$), and those in the 1 bl/s velocity group and 2 bl/s velocity group were significantly higher than in the control group and 2.5 bl/s velocity group ($p < 0.05$). The VT of coral trout was the widest in the 1 bl/s velocity group and the narrowest in the 2 bl/s velocity group. The numbers and sizes of goblet cells decreased with the increase in water flow velocity (Figure 5).

Table 3. Effect of water flow velocity on the index of midgut tissue in coral trout.

Item	Control	1 bl/s Water Flow Velocity	2 bl/s Water Flow Velocity	2.5 bl/s Water Flow Velocity
VL (μm)	498.13 ± 31.35 ^{ab}	523.97 ± 50.27 ^{a}	433.67 ± 27.69 ^{b}	443.2 ± 47.22 ^{b}
MS (μm)	110.88 ± 12.76 ^{c}	160.6 ± 16.83 ^{b}	235.57 ± 28.63 ^{a}	129.26 ± 6.27 ^{c}
VT (μm)	54.51 ± 6.64 ^{ab}	59.45 ± 7.71 ^{a}	48.77 ± 5.88 ^{b}	57.81 ± 3.43 ^{ab}

The values are average ± standard deviation of four replications (n = 6). Values with different letters show significant differences among the groups ($p < 0.05$), and those with no letters show no significant difference ($p > 0.05$).

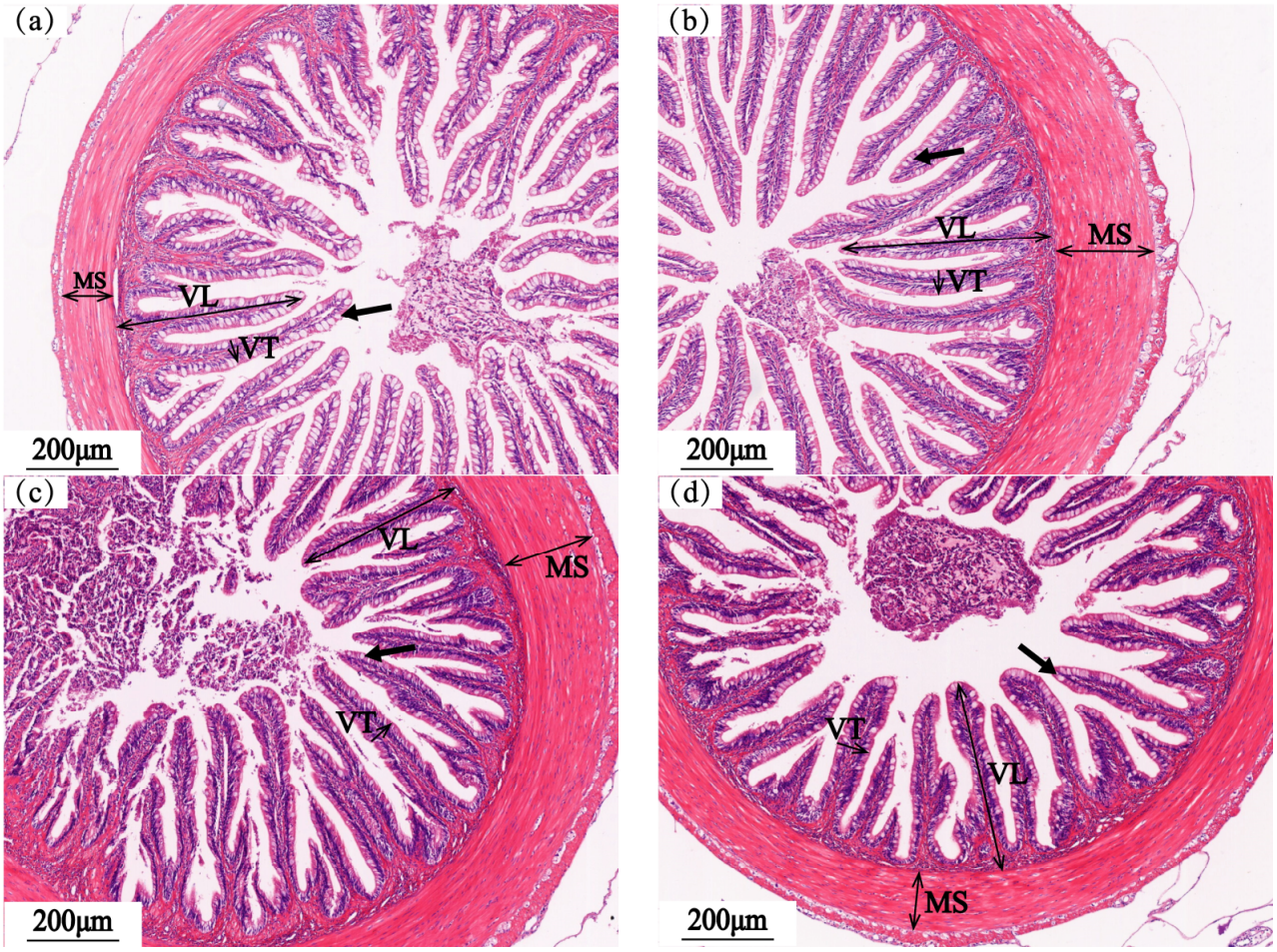

Figure 5. The effect of water flow velocity on the midgut structure of coral trout. (**a**) Hydrostatic control group: muscular thickness (MS), villus length (VL), and villus thickness (VT) were uniform, and there were many goblet cells (thick arrows); (**b**) 1 bl/s water flow velocity group: compared with the control group, the muscular layer was thicker, the villus length (VL) was longer, the villus thickness (VT) was not significantly different, and the number of goblet cells (thick arrows) was greater, but the size was relatively smaller; (**c**) 2 bl/s water flow velocity group: the muscular layer was thicker than in the control, the numbers and sizes of goblet cells (thick arrows) were significantly reduced, and some columnar epithelial cells in the mucosa layer were separated from the mucosa layer; (**d**) 2.5 bl/s water flow velocity group: muscular thickness (MS) was uniform, the number of goblet cells (thick arrows) was relatively small and the size was small.

3.4.3. Hindgut

In the hindgut tissue, the VL and MS of coral trout were significantly higher in the 1 bl/s water flow velocity group than in the control and other water flow velocity groups ($p < 0.05$). In terms of both MS and VT, the MS of coral trout in the 2.5 bl/s flow velocity group was the lowest, and there was no significant difference in VT among the 1 bl/s

water flow velocity group and the control group ($p > 0.05$, Table 4). At 2 bl/s water flow velocity, the goblet cells showed normal levels, whereas at 2.5 bl/s water flow velocity, the goblet cells were fragmented and unevenly distributed, with significantly reduced numbers compared to all other groups (Figure 6).

Table 4. Effect of water flow velocity on the index of hindgut tissue of coral trout.

Item	Control	1 bl/s Water Flow Velocity	2 bl/s Water Flow Velocity	2.5 bl/s Water Flow Velocity
VL (μm)	628.24 ± 43.82 ^{b}	838.13 ± 47.86 ^{a}	627.75 ± 68.6 ^{b}	684.7 ± 56.29 ^{b}
MS (μm)	185.87 ± 13.17 ^{b}	216.57 ± 25.83 ^{a}	178.45 ± 22.08 ^{b}	171.56 ± 7.89 ^{b}
VT (μm)	67.8 ± 7.26 ^{ab}	54.01 ± 8.76 ^{b}	64.15 ± 9.47 ^{ab}	69.99 ± 11.34 ^{a}

The values are average ± standard deviation of four replications ($n = 6$). Values with different letters show significant difference among the groups ($p < 0.05$), and those with no letters showed no significant difference ($p > 0.05$).

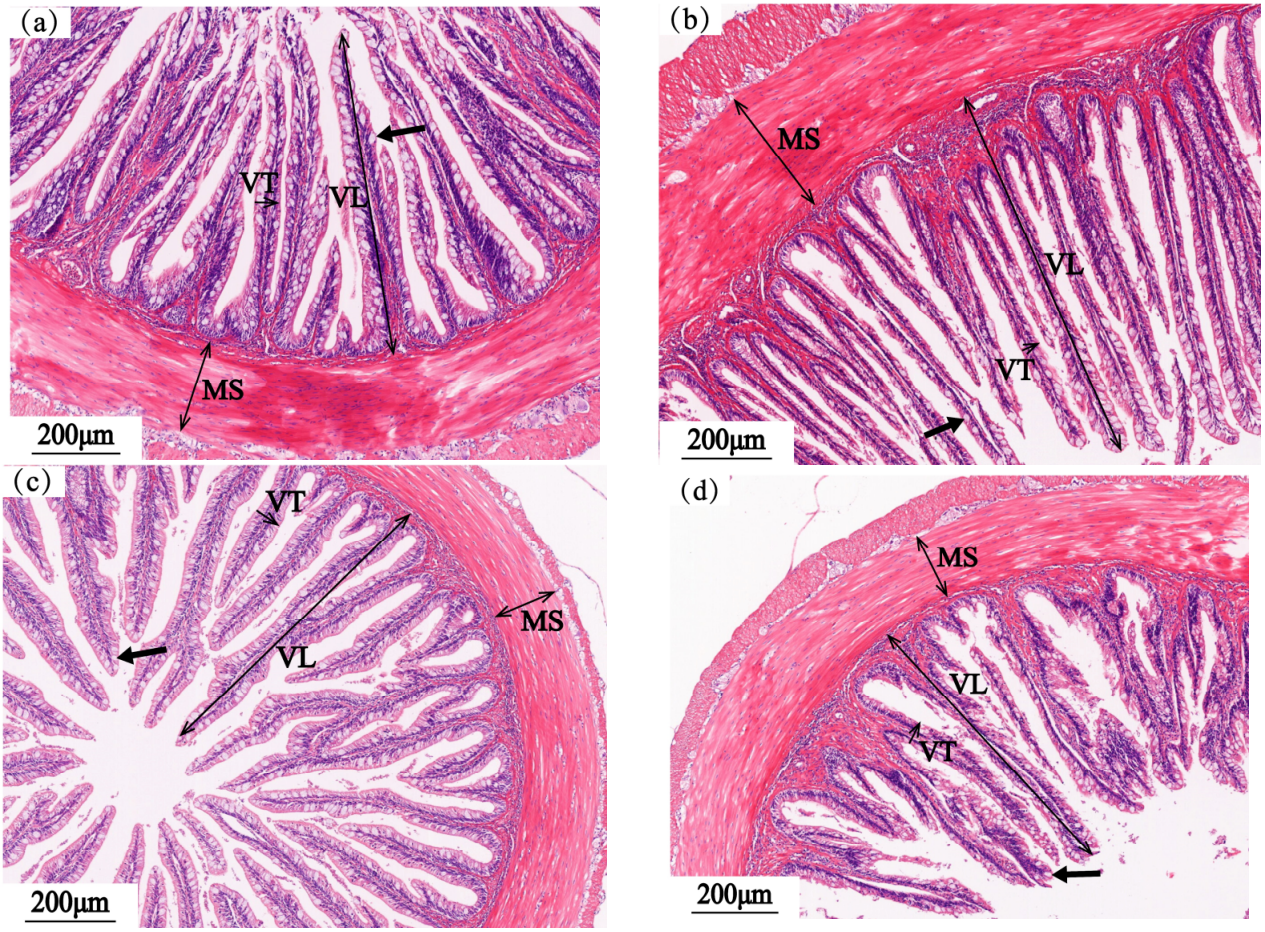

Figure 6. The effect of water flow velocity on the hindgut structure of coral trout. (**a**) Hydrostatic control group: muscular thickness (MS), villus length (VL), and villus thickness (VT) were uniform, and there were more goblet cells (thick arrows); (**b**) 1 bl/s water flow velocity group: the muscle layer was thicker than in the control, the villus was the longest, and the goblet cells (thick arrows) were relatively large; (**c**) 2 bl/s water flow velocity group: the muscle layer was the thinnest, and the goblet cells (thick arrows) were evenly distributed and numerous; (**d**) 2.5 bl/s water flow velocity group: some of the epithelial cells at the end of the villus fell off, and the goblet cells (thick arrows) were broken and unevenly distributed in size. The muscle layer was thicker, and the number of goblet cells (thick arrows) was significantly reduced compared with other groups.

4. Discussion

The different water flow velocities manifested significant differences in the growth performances of the fish. In a study by Wei [11], the highest SGR and WGR values were found in the 1 bl/s water flow velocity group of *Epinephelus coioides* (42.54 ± 0.62 g), and 2 bl/s water flow velocity negatively affected fish growth. Turbot (*Scophthalmus maximus*) (average body length 20.10 cm) achieved higher SGR values by swimming and exercising at 0.9 bl/s water flow velocity [12]. This is consistent with the results obtained in the present study, where the 1 bl/s water flow velocity group had the highest WGR and SGR values and the fastest growth. The desire to feed was enhanced when the current exercised the fish [13]. The SGR value of coral trout tended to decrease when the water flow velocity exceeded 1 bl/s, reaching a minimum at 2.5 bl/s water flow velocity. Goldfish (*Carassius auratus*) (10.1 ± 0.2 cm) that received high-water flow velocity exercise showed reduced feeding compared to the control [14]. Fish are capable of spontaneous swimming exercises at suitable water flow velocities, which helps to improve the efficiency of energy metabolism and accelerate their growth. At high-water flow velocities, the fish are forced to swim, and they fatigue faster, thus affecting their growth. At a high-water flow velocity (4 bl/s), *Schizothorax prenanti* (average body length 9.70 cm) allocated more energy to swimming and showed reduced growth performance [15].

It has been suggested that there are significant differences in the morphological characteristics of fish at different water flow velocities [16]. In the present experiment, however, there was no significant difference in the fatness of the fish among the groups, with the highest CF at 1 bl/s water flow velocity. The HSI value was also not significantly different, but the VSI ratio was, with the VSI in each water flow velocity group being smaller than that in the hydrostatic control group, and the VSI in the 2 bl/s water flow velocity group being the smallest. The reason for this is the difference in the thickness and mass of the fat envelope between the organs. Liu [17] pointed out that, as the water flow velocity increased, *Micropterus Salmoides* (8.13 ± 0.82 cm) broke down body fat to provide energy for maintained swimming movement, and the appropriate water flow velocity (5.0 bl/s) could reduce the excessive accumulation of fat in the fish, enhance the absorption of nutrients, and improve the quality of fish meat. However, when the water flow velocity exceeds the tolerance limit, the fish become fatigued, and no longer swims.

GLU metabolism is an important physiological metabolic process in animals. GLU can provide energy and carbon sources for the growth and metabolism of the body and meet the basic needs of the body's life activities [18]. In this experiment, the GLU concentration of the water flow velocity group was lower than that of the control group, and those in the 2 bl/s and 2.5 bl/s water flow velocity groups were significantly different from those in the control group, far below the average level. Under transport stress, the GLU concentration of silver pomfret (*Pampus argenteus*) increased significantly, and the breakdown of liver glycogen and muscle glycogen in these fish provided energy to ensure that the fish withstood the stress [19]. Different from the results of this experiment, the GLU concentration of black porgy (*Acanthopagrus schlegelii*) (6.75 ± 0.03 cm) increased at a high-water flow velocity (4.0 bl/s) [20]. The reason for this difference may be related to the swimming ability and living habits of fish. In another study, the GLU concentration of largemouth bass decreased with increasing water flow velocity [17]. Under acute hyperosmotic stress, the Chinese mitten crab (*Eriocheir sinensis*) undergoes corresponding adaptive physiological and biochemical changes, preferentially using GLU for energy supply to regulate osmotic pressure, and subsequently using nutrients, such as proteins [21]. At a high-water flow velocity (2.0 bl/s), tinfoil barbs (15.1 ± 1.35 cm) require more GLU to provide energy, which takes priority over protein and fat [22]. When GLU supply is inadequate, the organism derives additional energy from the breakdown of fat and protein to meet the needs of forced swimming to the detriment of nutrient accumulation in fish, and experimental fish undergo greater energy expenditure at high-water flow velocities (≥2 bl/s), the specific regulatory and metabolic mechanisms of which require further study.

LD is commonly considered to be the main metabolic product produced by muscles after exercise, and that which cannot be processed in the muscles diffuses and is released into the hemolymph and blood for absorption and use by other tissues [23]. LD concentrations in the blood of black porgy (19.37 ± 1.88 cm) increased with exercise time, and fatigue was positively correlated with LD concentration [24]. In the present study, LD concentration in the blood showed a trend of decreasing and then increasing as the water flow velocity increased. A 1 bl/s water flow velocity correlated with the lowest LD concentration. At water flow velocities above 2 bl/s, the coral trout swimming activity was shown to involve anaerobic exercise, with higher LD concentrations than in the hydrostatic control group, thus requiring additional energy to maintain it. Swimming at high-water flow velocities leads to more energy expenditure, which is consistent with the findings in grass carp (*Ctenopharyngodon Idella*) (16.83 ± 0.96 cm) [25].

Cortisol is an important hormone secreted by the fish's body in response to external stimuli and is often used as an indicator of the physiological response to stress in fish. When the external environment causes stress in fish, it triggers the release of corticotrophin-releasing hormone (CRH) by the hypothalamus, which stimulates the secretion of adreno-cortico-tropic-hormone (ACTH) from the anterior pituitary gland of the hypothalamus, which is transmitted to the interrenal tissues to aid in the production of corticosteroids. The degree of stress in fish is related to the duration and intensity of the stress [26]. Atlantic salmon (19.26 ± 0.08 cm) subjected to 24 weeks of exercise at different water flow velocities (0.5 to 2.5 bl/s) showed similar levels of COR across treatment groups [7]. The research reported that GLU and COR are usually elevated for an initial period following stress, and then gradually decrease until normal levels are restored due to the negative feedback mechanisms arising from homeostasis and adaptation of the hypothalamic–pituitary–interrenal axis [27]. In another study, chronic high-flow stress (4.0 bl/s) also had no significant effect on COR levels in the blood of largemouth bass (4.50 ± 0.36 cm) [28]. Under crowding stress, a trend was observed of increasing and then decreasing COR concentrations in the whole bodies of *Ancherythroculter nigrocauda* (2.71 ± 0.31 cm) [29]. In the present study, there were no significant differences in COR concentrations under different water flow velocity conditions, and the experimental fish showed a good adaptive capacity. In conclusion, although the fish species, stressors, and modes of action were different, no significant differences were seen in COR concentrations among the treatment groups as the time was extended, indicating that the fish had an excellent adaptive capacity to water flow velocity.

The intestine is an essential digestive organ for fish, and the level of digestive enzyme activity directly affects the digestion and absorption of bait in this fish. The protease, LPS, and AMS activities of Jiffy Tilapia (*Oreochromis niloticus*) (15.1 ± 0.21 cm) were significantly increased under prolonged water flow [30]. The AMS activity of juvenile *Rhynchocypris lagowskii* (2.21 ± 0.07 g) showed a trend of increasing and then decreasing with increasing water flow velocity, which is similar to the results of this experiment [31]. A 1 bl/s water flow velocity correlated with the highest AMS activity in the intestine of *Oreochromis niloticus*, and the α-AMS activities of the 2 bl/s and 2.5 bl/s water flow velocity groups were significantly lower than those of the control and 1 bl/s groups. The high-water flow velocity group showed a poor ability to absorb starch-containing feeds, reducing feeding motivation. The lower GLU concentration observed in the high-water flow velocity group in the present study may be partly due to the lower activity of α-AMS at this water flow velocity, which affects the digestion and absorption of feed. LPS was not affected by water flow velocity, and there were no significant differences among the groups.

In the intestine, the secretion ability of mucous cells increases from front to back, and the mucous digestion ability of the rectum is the strongest in the intestine, which is closely related to the physiological function of the rectum [32]. An increase in VL in the intestine can increase the contact area with food, thus expanding the absorption area and improving the digestive capacity of the intestine [33]. The intestinal VL of experimental fish in the 1 bl/s water flow velocity group was significantly lower than that of other groups, possibly

due to the fact that stimulation via water flow velocity weakens the digestive ability of the foregut, enhancing the digestive ability of the midgut and hindgut, and enabling them to secrete more goblet cells to promote food digestion. The specific causes need further experimental investigation. In this experiment, the VL, MS, and VT values in the midgut of each group were lower than those in the foregut and hindgut, and the relative absorption capacity of the midgut was poor. Therefore, the absorption of nutrients by the coral trout is mainly concentrated in the foregut and hindgut. Under stimulation by water flow velocity, the MS is increased to a certain extent, which can increase the elasticity of the intestine and promote food peristalsis. At 1 bl/s water flow velocity, the structural parameters of the foregut were slightly lower than those of the control group and other water flow velocity groups; further, both the midgut and hindgut were superior to those of other groups. In general, the intestinal structure of the 1 bl/s water flow velocity group was better than that of other water flow velocity groups, and its digestive enzyme activity was also higher than that of other water flow velocity groups. Medium and high-water flow velocities (2 bl/s and 2.5 bl/s) have a specific adverse effect on the shape and quantity of goblet cells, even causing cell breakage and other conditions, and thus have an inhibitory effect on digestive and immune functions. This is also in line with the lower digestive enzyme activity in these groups compared with the control.

In this experiment, the SGR and WGR values of coral trout showed high consistency with intestinal digestive enzyme activity. At a water flow velocity of 2.5 bl/s, the digestive enzyme activity of coral trout is weakened, resulting in a decrease in growth performance, while the GLU content also reflects nutritional deficiencies from a lateral perspective. At this water flow velocity, there was no significant impact on VT, VL, or MS. However, the reductions in the number of goblet cells and the structural changes are the main reasons for the reduced digestive capacity that was observed.

5. Conclusions

The results show that under water velocity stress, the growth indicators, serum biochemical indicators, intestinal digestive enzyme activity, and intestinal structure of coral trout have changed. High-water flow velocity ($\geq$2 bl/s) stress can lead to a decrease in GLU levels and an increase in LD content in fish, disrupting their normal physiological homeostasis. The high-water flow velocity has an inhibitory effect on the α-AMS activity of the coral trout and reduces the number of goblet cells, altering the intestinal structure and weakening the digestive capacity of the gut, ultimately leading to a reduction in SGR and WGR. This study shows that coral trout are sensitive to water flow velocity, and high flow velocity can exacerbate physiological disorders, weaken digestive enzyme activity, and alter the intestinal structure in this species. Therefore, in actual RAS aquaculture, the water flow speed should be controlled within 1 bl/s, and the impact of high-water flow velocity stress on this species' physiology and intestinal digestion should be avoided as much as possible to achieve a better aquaculture environment.

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