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Research Article

Effect of Stocking Density on Culture Efficiency and Physiological Indicators of Largemouth Bass (*Micropterus salmoides*) under Recirculating Water Conditions in Land-Based Round Ponds

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To identify the optimal stocking rate of *Micropterus salmoides* culture in a land-based round pond recirculating aquaculture system, five stocking densities were tested, i.e., D1–D5 with 60, 70, 80, 90, and 100 fish/m^2, respectively. The water temperature of the experimental pond was 20.0–26.5°C. The effects of stocking density on culture productivity and physiological indexes of *M. salmoides* (initial body weight 50.83 ± 7.28 g) were examined. The D1 and D5 treatments exhibited significantly improved growth performance. The population weight gain in the D5 treatment was the highest, which increased by 63.73%, 52.82%, 60.93%, and 45.08%, compared with groups D1–D4 ($P < 0.05$, each), and the feed conversion ratio was the lowest, which was decreased by 5.65%, 12.03%, 26.42%, and 21.48% compared with groups D1–D4. The levels of crude protein, essential amino acids, and total free amino acids in muscle tissue of group D3 were higher than those in the other groups. Stocking density did not differ significantly on muscle fatty acid composition ($P > 0.05$, each). The activities of serum AKP and LZM and liver SOD, AKP, and LZM in group D5 were significantly higher than those in the other groups. Thus, 100 fish/m^2 was the optimal stocking density of M. salmoides at a size stage of 50.83~130.13 g in the circulating water of land-based round ponds.

1. Introduction

Aquaculture provides animal-based food and bioenergy for the world's growing population. In 2020, the output of aquaculture products in China reached 53,944,100 tons, of which 33,030,050 tons of freshwater products, accounting for 61.2% [1]. Pond culture, as the primary mode of freshwater culture in China, reached an area of 2,644,726 ha [2]. However, owing to the rapid development of aquaculture, land and water resources are becoming increasingly scarce, and environmental pollution is aggravating. Thus the conflict between economic growth and ecological protection has become a severe issue in current aquaculture [3]. To achieve "environmental protection, improved quality and efficiency, and rich fishermen," it is necessary to promote the transformation of aquaculture approaches. Land-based circular-pond circulating water aquaculture was a breakthrough in "adjusting the structure and transforming the mode" of the fishery. Land-based round pool circulating water aquaculture is a new land-based "cylindrical semi-closed facilities" aquaculture intensive and efficient aquaculture method that makes full use of the advantages of nonred line arable land and rich surface and groundwater resources and has the advantages of occupying less land. Further, it is not affected by topography and terrain, does not interfere with the nature of the land, offers a high degree of intensive intelligence, and has a robust and self-cleaning ability of the aquaculture system. This system may help

address the current bottleneck of fisheries development through promoting more sustainable and high-quality fishery and providing high-quality aquatic products for consumers.

The largemouth bass (*Micropterus salmoides*; order Perciformes, family Centrarchidae) also known as California perch, is a freshwater fish native to North America. It was introduced to mainland China after the 1980s [4]. It has been widely cultivated owing to its rich nutritional value, easy catchability, fast growth rate, delicious meat, and wide range of temperature acceptance. Thus, it has become one of the most economically important freshwater fish and a pre-eminent species for intensive aquaculture in freshwater ponds [5]. Current largemouth bass cultivation in China faces challenges including the single farming model and the imbalance of farming areas that cause substantial market price fluctuation. Largemouth bass breeding is mainly based on pond monoculture, and pond breeding is generally performed using free-range breeding strategies of high density, high feeding volume, and high water change rate, which interfere with the ecological balance and self-purification ability of the pond, thus entailing various problems such as deterioration of breeding water quality, frequent emergence of diseases, and antibiotic abuse [6], resulting in low breeding success and high breeding risk. From the perspective of ecological protection, breeding wastewater from pond cultures of largemouth bass is currently discharged directly without any treatment, and when issues occur such as water quality deterioration, fish morbidity, etc., breeders typically perform numerous water changes [7]. This facilitates the direct entry of breeding wastewater into the natural environment, which severely affects the ecosystems of the receiving water bodies. To address these problems, options such as pond runway-type recirculating water culture and container recirculating water culture were investigated, which showed promising results; however, issues such as high operating costs and varying economic benefits remain to be resolved. Therefore, identifying novel approaches for sustainable aquaculture is a matter of urgency.

Stocking density is an essential factor affecting the growth and development of fish and the pressure of carrying fish in aquaculture water. Determining the optimal density is thus a prerequisite for ensuring the economic feasibility of fish production [8]. However, commercial production must also consider other factors such as animal growth performance, stress, health, and immune capacity [9]. In some fish species, positive and negative effects of different stocking densities on fish growth have been reported [10–12]. Excessive stocking density may cause chronic stress, inhibit fish growth, and reduce the welfare of farmed fish [13, 14]. By contrast, below-optimum densities may result in poor growth and, thus, lower overall productivity due to underutilization of available resources [15]. Therefore, exploring the optimal stocking density suitable for the growth, physiological health, yield increase, and efficiency of cultured fish is vital. In addition, based on not affecting the above parameters, increasing stocking density can result in higher yields. With the development of intensive aquaculture, increasing the stocking density has become a way to optimize fish production [16]. For example, in a study of broad-bodied golden leech culture trials, the average net production value of the high-density group (100 tails/m^2, ¥69,791/hm^2) was markedly higher than that of the low-density group (50 tails/m^2, ¥57,015/hm^2), with a 47.59% increase in total production and a 22.28% increase in efficiency [17]. Intensive aquaculture technology ensures that the space in the production system can be fully used by stocking a particular area with the maximum number of fish to improve production yield and efficiency. While excessive breeding densities can create competitive pressure for feeding and space, leading to impaired growth and immunity in cultured subjects [18], cost-effective production should ensure maximum yield with minimal physiological stress and disease incidence [19].

Land-based, round pool circulating water aquaculture is a new method of intensive inland farming. So far, there are few mature models to draw on in terms of stocking density, feeding techniques, and water quality management. Considering the problems of low growth efficiency, high feed energy consumption, frequent disease emergence, and inadequate control of the aquaculture water environment caused by unreasonable stocking density in the current land-based round pond culture system, we studied the effects of various stocking densities on breeding efficiency and health of M. salmoides using a land-based round pond culture system. Our results can provide technical references for the healthy development of land-based round pond aquacultures.

2. Materials and Methods

2.1. Materials. The test ponds were 10 productive land-based round ponds at the breeding base in Dahua County, Guangxi. The pools were pot-bottom-shaped, with a diameter of 8.2 m, a wall height of 1.5 m, and a water depth of 1.4 m. The horizontal height from the bottom of the wall to the drainage outlet at the "bottom of the pot" was 0.5 m, and the area was 52.78 m^2. Intake, drainage, and gas supply (gas control) facilities were well supported. The water used for breeding was reservoir irrigation water plus pumped groundwater, and the water temperature of the fish pond ranged from 20.0 to 26.5°C during the experiment. We used *M. salmoides* with an initial average body mass of 50.83 ± 7.28 g and an initial average body length of 12.64 ± 0.87 cm. The experimental diet was Rongchuan brand California perch expanded compound feed (crude protein ≥ 50.0%, crude ash ≤ 18.0, 0.8 ≤ calcium ≤ 4.0%, moisture ≤ 10.0%, crude fiber ≤ 3.5%, total phosphorus ≥ 1.2%, crude fat ≥ 5.0%, lysine ≥ 3.1%).

2.2. Experimental Design and Culture Management. Five stocking densities were used, i.e., D1 (60 fish/m^2), D2 (70 fish/m^2), D3 (80 fish/m^2), D4 (90 fish/m^2), and D5 (100 fish/m^2), with replicates in each group. Feed was provided twice per day (at approximately 8:00 and 17:00). The water was exchanged approximately 1 h after each feeding

and was discharged once at the same time. At each water change, approximately 1/10 of the original pool water was replaced from the start of the experiment to day 30, 1/6 of the original pool water from day 31 to 46, and 1/3 of the original pool water from day 47 to 63. During the breeding period, water was continuously fed into the inlet of each pool, and the inlet flow rate of each pool was approximately 1.17 L/s from 8:00 to 20:00 and 0.68 L/s from 20:00 to 8:00. The drainage facilities of each pool were based on the theory of communicating vessels. The inlet water flow rate was the natural drainage (microflow) flow rate. The whole process of uninterrupted aeration and oxygenation was contrived to maintain the dissolved oxygen level in the pool water at approximately 6.3 mg/L. An oxygenation process and "push flow" devices to maintain the flow of water in the pool were used. Apart from stocking density, the culture management methods were the same in each pond. The duration of the experiment was 63 days.

2.3. Sample Collection. Thirty fish were randomly selected on the day of stocking to determine the full length, body length, and body mass. At the end of the rearing test, 30 fish were randomly selected from each pool to determine the main growth indexes; 10 fish were randomly selected and anesthetized with MS-222 (130 mg/L), and blood was drawn from the caudal vein below the anal fin of each sample using a syringe of approximately 5 mL volume. The blood samples were incubated at 4°C for 4 h, and the supernatant was centrifuged for 10 min (4°C, 5,000 r/min) to be used for serum antioxidant assays. Dorsal muscle tissue was collected for routine nutrient, amino acid, and fatty acid analysis; the intestine and liver were collected for digestive enzyme and antioxidant assays.

2.4. Growth Indicators. Weight gain rate (WGR), specific growth rate (SGR), daily growth rate in body length, hepatosomatic index (HIS), viscerosomatic index (VSI), condition factor (CF), feed conversion rate (FCR), survival rate (SRe), and coefficient of variation were assessed according to the following equations:

$$\begin{aligned}
\mathrm{WGR}\ (\%) &= 100 \times \frac{(W_t - W_0)}{W_0},\\
\mathrm{SGR}\ (\%/d) &= 100 \times \frac{(\mathrm{LnW}_t - LnW_0)}{t},\\
\text{Body length daily growth rate}\ (\%) &= 100 \times \frac{(L_t - L_0)}{(L_0 \times t)},\\
\mathrm{HIS}\,(\%) &= \frac{100 \times W_h}{W_t},\\
\mathrm{VSI}\,(\%) &= \frac{100 \times W_v}{W_t},\\
\mathrm{CF}\left(\frac{g}{\mathrm{cm}^3}\right) &= \frac{100 \times W_t}{(L_t)^3},\\
\mathrm{FCR} &= \frac{\text{weight of fed feed}}{\text{weight gain of fish} \times 100\%},\\
\mathrm{SR}\ (\%) &= 100 \times \frac{\text{survival number}}{\text{initial total number of fish}},\\
\text{Coefficient of variation} &= \frac{\mathrm{SD}}{\mathrm{Mean} \times 100\%},
\end{aligned} \tag{1}$$

where W_t is the final mean body mass (g); W_0 is the initial mean body mass (g); W_h is the weight of the liver (g); W_v is the weight of the viscera; L_t is the final mean body length (cm); L_0 is the initial mean body length (cm); t is the number of breeding test days (d); SD is the standard deviation of the final body mass of fish in the same pool; and Mean is the mean of the final body mass of fish in the same pool.

2.5. Nutrient Composition. Crude protein content was determined using the Kjeldahl method (GB5009-2010, semimicro Kjeldahl method); crude fat was determined using the Soxhlet extraction method (GB5009.6-2003); crude ash was determined by cauterized mass method (GB5009.4-2010, cauterized at 550° in a muffle furnace); crude moisture was determined by drying (GB5009.3-2010, 105°C drying

method); free amino acids were assessed using phenyl isothiocyanate derivatization-high performance liquid chromatography [20]. Fatty acids were determined using the GB/T5009.168-2016 method.

2.6. Digestive Enzyme Activity. The intestinal α-amylase (AMS), lipase (LPS), and trypsin (TPS) activities were measured using respective kits produced in Nanjing. Sample pretreatment, reagent preparation, and sample determination were carried out strictly according to the instructions.

2.7. Antioxidant Assays. Superoxide dismutase (SOD), alkaline phosphatase (AKP), catalase (CAT), malondialdehyde (MDA), and lysozyme (LZM) activities in the serum and liver were measured using respective kits developed in Nanjing, China. Lysozyme activities were measured using the respective kits. Sample pretreatment, reagent preparation, and measurements were carried out according to the manufacturer's instructions.

2.8. Data Analyses. The experimental data were compiled and processed using Microsoft Excel 2019, and Shapiro–Wilk W and Levene's tests were performed using SPSS 26.0 to verify normality and homogeneity of variances. If the variance was consistent ($P > 0.05$), differences were tested using a one-way analysis of variance (ANOVA), and significant outcomes were followed by multiple comparison analyses using least-significant difference tests. When the variance was inconsistent ($P < 0.05$), multiple comparisons were performed using Dunnett's T3 analysis. Statistical significance is reported at $P < 0.05$. Data are shown as means ± standard deviation.

3. Results

3.1. Growth Performance and Breeding Efficiency

3.1.1. Growth Performance. The growth indices of *M. salmoides* under different stocking densities are shown in Table 1. The final weight and final body length of *M. salmoides* reached their maxima in the D1 and D5 treatments, respectively, and they were significantly higher than those of the D3 and D4 treatments; the weight gain rate and specific growth rate of group D1 were the highest, followed by those of groups D5 and D2, respectively; however, the difference between the groups was not significant, and the weight gain rates of groups D1, D5, and D2 were significantly higher than those of groups D3 and D4. The weight gain rate of groups D1, D5, and D2 was significantly higher than that of groups D3 and D4; group D5 had the highest daily growth rate of body length, which was significantly higher than that of groups D3 and D4. Hepatosomatic index, viscerosomatic index, and condition factor did not differ significantly among the groups. The fish grew fastest in the D1 and D5 treatments, with specific growth rates of 1.49%/day and 1.44%/day, respectively, which increased by 8.76%, 20.16%, and 21.14% in group D1 compared with groups D2 to D4, and by 5.11%, 16.13%, and 17.07% in group D5 compared with groups D2 to D4, respectively.

3.1.2. Culture Efficiency. The total production and group weight gain of *M. salmoides* in group D5 were the highest (Table 1) and significantly higher than those in all other groups under the same area and water volume; the feed coefficient in group D5 was the lowest, i.e., 26.4% lower than that in group D3, which was, however, not significant. Survival rates did not differ significantly between groups. The coefficient of variation of group D4 was significantly lower than that of group D3, and no significant difference with other groups was observed.

A stocking density of 100 fish/m^2 (group D5) was optimal, with a body mass gain of 346.28 kg, which was 63.73%, 52.82%, 60.93%, and 45.08% higher than those of groups D1, D2, D3, and D4, respectively. Feed energy consumption was the lowest in D5, with feed coefficients that were 5.65%, 12.03%, 26.42% and 21.48% lower than those of groups D1, D2, D3, and D4, respectively.

3.2. Nutritional Composition

3.2.1. Conventional Nutrient Composition. The crude muscle protein content of group D3 was significantly higher than that of groups D1 and D4 (Table 2), and there was no significant difference with other groups. Crude fat and moisture content did not differ significantly between treatments. The ash content of group D4 was significantly lower than that of group D2, whereas no other significant difference was observed. The content of liver fat tended to decrease with the increase of the stocking density and was the lowest in the D5 group, which was significantly decreased compared to the D1 and D2 groups.

3.2.2. Free Amino Acid Content in Muscle Tissue. Fifteen free amino acids were detected in muscle tissue of *M. salmoides*, including eight essential and seven nonessential amino acids (Table 3). The histidine (bitter) content of each test group exceeded the threshold value ($20\,mg^{-100}{\cdot}g^{-1}$) and showed a decreasing trend with the increase of the stocking density, reaching the lowest in group D5; however, the difference between groups was not significant. Essential and total free amino acid levels were the highest in group D3 and the lowest in group D1 ($P > 0.05$); fresh amino acids content was the highest in group D2 and the lowest in group D4, and no other significant difference between groups was observed. Essential and total free amino acid levels were slightly higher in group D3 than in the other groups, which was not significant.

3.2.3. Muscle and Liver Fatty Acid Content. A total of 26 fatty acids were detected in the muscle tissue of *M. salmoides*, including 11 saturated fatty acids (SFA), 7 monounsaturated fatty acids (MUFA), 7 polyunsaturated fatty acids (PUFA), and 3 highly unsaturated fatty acids (HUFA) (Table 4). Stocking

Table 1: Growth and production indexes of *M. salmoides* under different stocking densities.

Parameters	D1	D2	D3	D4	D5
Final weight (g)	130.13 ± 7.78^{a}	120.39 ± 1.57^{ab}	111.50 ± 9.10^{b}	110.44 ± 1.21^{b}	126.38 ± 8.24^{a}
Final body length (cm)	17.47 ± 0.22^{a}	17.12 ± 0.09^{ab}	16.71 ± 0.50^{b}	16.57 ± 0.01^{b}	17.54 ± 0.22^{a}
Weight gain rate (WGR, %)	156.01 ± 15.30^{a}	136.85 ± 3.08^{ab}	119.36 ± 17.90^{b}	117.28 ± 2.38^{b}	148.62 ± 16.22^{ab}
Special growth rate (SGR, %/d)	1.49 ± 0.09^{a}	1.37 ± 0.02^{ab}	1.24 ± 0.13^{b}	1.23 ± 0.02^{b}	1.44 ± 0.10^{ab}
Body length daily growth rate (BLG, %/d)	0.29 ± 0.02^{a}	0.26 ± 0.01^{ab}	0.22 ± 0.05^{b}	0.20 ± 0.01^{b}	0.30 ± 0.02^{a}
Hepatosomatic index (HIS, %)	1.25 ± 0.12	1.41 ± 0.24	1.43 ± 0.01	1.42 ± 0.19	1.46 ± 0.24
Viscerosomatic index (VSI, %)	11.73 ± 4.97	13.50 ± 0.52	11.28 ± 0.63	12.43 ± 0.59	12.89 ± 2.24
Condition factor (CF, g/cm$^{3)}$	2.44 ± 0.05	2.40 ± 0.01	2.39 ± 0.02	2.43 ± 0.03	2.34 ± 0.06
Total production (kg)	373.13 ± 41.22^{c}	415.17 ± 14.43^{bc}	430.70 ± 47.88^{bc}	481.15 ± 5.20^{b}	645.68 ± 54.97^{a}
Group weight gain (kg)	211.49 ± 41.22^{b}	226.59 ± 14.43^{b}	215.18 ± 47.88^{b}	238.69 ± 5.20^{b}	346.28 ± 54.97^{a}
Feeding amount (kg)	259.03 ± 13.47^{e}	299.50 ± 3.18^{d}	333.55 ± 0.99^{c}	356.05 ± 6.58^{b}	401.53 ± 7.88^{a}
Feed conversion rate (FCR)	1.24 ± 0.18	1.33 ± 0.10	1.59 ± 0.36	1.49 ± 0.01	1.17 ± 0.16
Survival rate (SRI, %)	90.03 ± 4.58	92.94 ± 2.02	90.99 ± 2.70	91.33 ± 0.01	91.85 ± 2.22
Coefficient of variation (CV)	0.20 ± 0.02^{ab}	0.18 ± 0.00^{ab}	0.20 ± 0.04^{a}	0.15 ± 0.00^{b}	0.19 ± 0.00^{ab}

Note. There was no lowercase letter in the shoulder label of peer data or no significant difference in the expression of the same lowercase letter ($P > 0.05$). Different lowercase letters indicated significant differences ($P < 0.05$). The following table was the same.

Table 2: Effect of stocking density on the nutrient composition of *M. salmoides* (%, wet weight).

Parameter	D1	D2	D3	D4	D5
Crude protein	19.56 ± 1.24^{b}	20.71 ± 0.35^{ab}	21.87 ± 0.12^{a}	19.91 ± 0.33^{b}	20.99 ± 0.33^{ab}
Crude fat	1.35 ± 0.31	1.69 ± 0.73	2.28 ± 0.91	1.37 ± 0.02	1.65 ± 0.31
Moisture	78.19 ± 0.11	77.69 ± 0.45	77.51 ± 0.62	78.84 ± 1.00	78.02 ± 0.16
Ash	1.28 ± 0.06^{ab}	1.40 ± 0.01^{a}	1.33 ± 0.04^{ab}	1.22 ± 0.08^{b}	1.35 ± 0.11^{ab}
Liver fat	5.08 ± 0.65^{a}	4.93 ± 0.01^{a}	3.94 ± 0.43^{ab}	3.82 ± 0.15^{b}	3.77 ± 0.35^{b}

Table 3: Effect of stocking density on free amino acids in muscle tissue of *M. salmoides* (mg·100 g^{-1}, wet weight).

Amino acids	D1	D2	D3	D4	D5
Essential amino acid					
Isoleucine	1.98 ± 0.21	1.45 ± 0.05	1.91 ± 0.90	1.42 ± 0.42	1.74 ± 0.14
Leucine	10.75 ± 1.19	11.33 ± 0.98	13.45 ± 0.36	11.98 ± 2.49	12.41 ± 1.48
Methionine	2.94 ± 0.17	3.44 ± 0.27	4.27 ± 0.04	4.25 ± 1.63	3.92 ± 0.68
Histidine	41.34 ± 11.49	40.80 ± 2.58	40.64 ± 16.64	40.78 ± 0.62	33.99 ± 9.14
Threonine	11.36 ± 0.98	12.43 ± 0.39	13.49 ± 2.77	12.54 ± 1.82	14.61 ± 3.63
Valine	3.58 ± 0.69	3.60 ± 0.31	4.20 ± 1.07	3.39 ± 1.42	4.58 ± 0.79
Phenylalanine	2.59 ± 0.05	3.13 ± 0.61	4.00 ± 0.63	3.72 ± 1.75	3.78 ± 1.15
Lysine	9.91 ± 1.83	9.82 ± 0.47	11.47 ± 1.54	13.36 ± 6.73	9.84 ± 1.66
Nonessential amino acids					
Asparticacid*	8.93 ± 6.01	5.05 ± 0.25	8.10 ± 0.33	8.64 ± 0.24	9.29 ± 3.15
Glutamic acid*	15.07 ± 0.97	18.61 ± 1.28	22.45 ± 6.34	18.53 ± 8.42	21.38 ± 4.90
Serine	8.51 ± 0.97	9.24 ± 1.88	11.08 ± 2.28	9.20 ± 0.14	8.56 ± 0.39
Glycine*	57.77 ± 1.55	64.42 ± 24.09	52.28 ± 23.74	44.19 ± 3.70	37.69 ± 9.16
Alamine*	28.61 ± 1.07	36.18 ± 2.18	35.87 ± 3.43	29.78 ± 6.90	35.68 ± 6.06
Proline	5.78 ± 1.57	8.97 ± 0.31	11.12 ± 4.33	10.31 ± 4.70	14.21 ± 5.25
Tyrosine	2.49 ± 0.01	2.77 ± 0.22	2.76 ± 0.03	2.78 ± 0.75	2.34 ± 1.17
Σ FAA	211.63 ± 14.83	231.23 ± 26.86	237.11 ± 50.42	214.88 ± 20.60	214.01 ± 12.16
Σ EAA	84.47 ± 12.81	86.00 ± 2.14	93.44 ± 16.86	91.45 ± 3.43	84.86 ± 0.40
Σ UAA	110.38 ± 4.56	124.25 ± 22.93	118.70 ± 26.98	101.14 ± 11.86	104.04 ± 4.95

Note. FAA, free amino acids; EAA, essential amino acids; UAA, umami amino acids*.

density significantly affected the levels of lambda (C10:0), pentadecanoic (C15:0), and behenic (C22:0) acids, showing a decreasing trend with increasing stocking density; octanoic (C8:0); and lauric (C12:0) acids were detected only in group D5.

Stocking density had no significant effect on the composition of saturated fatty acids (∑SFA), monounsaturated fatty acids (∑MUFA), polyunsaturated fatty acids (∑PUFA), and highly unsaturated fatty acids (∑HUFA) in fish muscle.

Table 4: *M. salmoides* muscle fatty acid content at the various stocking densities (%).

Fatty acid	D1	D2	D3	D4	D5
C8:0	—	—	—	—	0.03 ± 0.4
C10:0	0.24 ± 0.06^{a}	0.16 ± 0.04^{ab}	0.18 ± 0.06^{ab}	0.14 ± 0.03^{ab}	0.07 ± 0.09^{b}
C12:0	—	—	—	—	0.02 ± 0.02
C14:0	1.01 ± 0.01	1.14 ± 0.36	1.33 ± 0.09	1.14 ± 0.15	1.10 ± 0.003
C15:0	0.05 ± 0.01^{b}	0.19 ± 0.07^{a}	0.22 ± 0.02^{a}	0.16 ± 0.05^{a}	0.18 ± 0.01^{a}
C16:0	18.13 ± 0.09	17.14 ± 0.24	18.12 ± 0.16	17.47 ± 1.11	18.17 ± 0.14
C17:0	0.18 ± 0.01	0.17 ± 0.04	0.20 ± 0.06	0.21 ± 0.03	0.17 ± 0.03
C18:0	6.01 ± 0.12	5.84 ± 2.05	5.23 ± 0.71	5.11 ± 0.33	5.28 ± 0.22
C20:0	0.24 ± 0.01	0.23 ± 0.01	0.17 ± 0.04	0.22 ± 0.01	0.23 ± 0.07
C22:0	0.18 ± 0.01^{a}	0.11 ± 0.01^{b}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}
C24:0	3.72 ± 0.28	3.53 ± 0.76	2.87 ± 0.31	3.20 ± 0.55	3.75 ± 0.02
∑SFA	29.75 ± 0.20	28.52 ± 2.69	28.33 ± 1.18	27.72 ± 0.45	29.09 ± 0.33
C16:1	1.73 ± 0.05	1.93 ± 0.88	2.59 ± 0.54	2.11 ± 0.59	2.44 ± 0.19
C17:1	0.05 ± 0.001	0.04 ± 0.05	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.01
C18:1n9t	0.10 ± 0.01	0.10 ± 0.01	0.14 ± 0.03	0.10 ± 0.03	0.14 ± 0.00
C18:1n9c	16.19 ± 0.61	17.25 ± 5.22	20.77 ± 2.22	17.90 ± 1.58	20.51 ± 1.27
C20:1	0.49 ± 0.01	0.48 ± 0.12	0.54 ± 0.14	0.44 ± 0.05	0.50 ± 0.02
C22:1n9	0.35 ± 0.002	0.33 ± 0.17	0.26 ± 0.08	0.25 ± 0.05	0.24 ± 0.06
C24:1	0.53 ± 0.27	0.19 ± 0.06	0.17 ± 0.04	0.22 ± 0.08	0.19 ± 0.14
∑MUFA	19.44 ± 0.94	20.32 ± 6.05	24.55 ± 2.98	21.08 ± 2.24	24.10 ± 1.58
C18:2n6c*	23.32 ± 0.79	26.16 ± 3.26	26.11 ± 0.29	26.13 ± 0.69	23.81 ± 0.60
C18:3n6*	0.24 ± 0.11	0.49 ± 0.11	0.43 ± 0.07	0.34 ± 0.33	0.57 ± 0.01
C18:3	1.69 ± 0.06	2.10 ± 0.59	2.15 ± 0.02	2.07 ± 0.06	1.88 ± 0.07
C20:2	0.81 ± 0.00	0.75 ± 0.14	0.72 ± 0.07	0.79 ± 0.06	0.76 ± 0.08
C20:3n6	0.43 ± 0.03	0.35 ± 0.05	0.44 ± 0.02	0.49 ± 0.07	0.57 ± 0.21
C20:3n3	1.60 ± 0.06	1.34 ± 0.70	1.06 ± 0.15	1.32 ± 0.14	1.27 ± 0.31
C22:6n3	22.71 ± 0.48	19.96 ± 6.19	16.22 ± 1.22	20.05 ± 2.10	17.95 ± 0.38
∑PUFA	50.81 ± 1.14	51.15 ± 3.34	47.13 ± 1.80	51.19 ± 2.69	46.81 ± 1.25
∑HUFA	24.75 ± 0.52	21.65 ± 6.94	17.73 ± 1.34	21.86 ± 2.31	19.79 ± 3.51
n-3/n-6	1.01 ± 0.004	0.81 ± 0.35	0.64 ± 0.04	0.79 ± 0.07	0.77 ± 0.004

Note. ∑SFA indicates total saturated fatty acids, ∑MUFA indicates total monounsaturated fatty acids, ∑PUFA indicates total polyunsaturated fatty acids, and ∑HUFA indicates highly unsaturated fatty acids. epa (C20:5n3), eicosapentaenoic acid; DHA (C22:6n3), docosahexaenoic acid. *Labeled as essential fatty acids: C18:2n6c, linoleic acid; C18:3n6, gamma linolenic acid.

3.3. Digestive Enzyme Activity. The activity of intestinal digestive enzymes in *M. salmoides* at the various stocking densities is shown in Table 5. AMS activity decreased significantly with the increase of stocking density. The highest activity of trypsin (TPS) was significantly higher in group D2 than in the other groups, while TPS activity was also significantly higher in groups D3, D4, and D5 than in group D1.

3.4. Antioxidation

3.4.1. Serum Antioxidant Properties. The serum antioxidant properties of *M. salmoides* at different stocking densities are shown in Table 6 and Figure 1. The serum SOD activity was the highest in group D2, which was significantly higher than that in groups D1, D4, and D5, while no other significant difference was observed. AKP activity was the highest in group D3, followed by group D1, and it was significantly higher in both groups than in all other groups. CAT activity was significantly higher in group D3 compared to groups D1, D4, and D5. MDA activity was significantly higher in group D1 compared to other groups. LZM activity was the highest in group D5, followed by group D4, which was significantly higher compared to groups D1 and D3. Serum SOD, AKP, and CAT activities (i.e., the serum antioxidant potentials) were highest in group D3, followed by those in group D5.

3.4.2. Antioxidant Properties of the Liver. The antioxidant properties of livers of *M. salmoides* at different stocking densities are shown in Table 7 and Figure 2. SOD activity in the D5 group was significantly higher than that in groups D1, D2, and D4, and it was increased by 9.87%, 18.51%, 5.78%, and 21.41%, respectively, compared with groups D1–D4. AKP activity in group D5 was significantly higher than that in the other groups, and it was increased by 93.86%, 73.46%, 81.48%, and 50.92%, respectively, compared to groups D1–D4. CAT activity reached a maximum in group D5 ($P > 0.05$). The MDA levels increased with the increase of the stocking density, and they were significantly lower in groups D1 and D2 than in group D5, and there was no significant difference between the other groups. The highest LZM activity occurred in group D5, followed by that in group D3, and both groups showed a significant increase compared with groups D1, D2, and D4. Stocking density significantly affected the liver SOD, AKP, and LZM activities and MDA content, and the antioxidant properties of the liver were most enhanced in the D5 group.

Table 5: Intestinal digestive enzyme activities of *M. salmoides* at different stocking densities.

Digestive enzymes	D1	D2	D3	D4	D5
AMS (U/mg prot)	0.38 ± 0.01^{a}	0.28 ± 0.02^{ab}	0.27 ± 0.07^{ab}	0.17 ± 0.06^{b}	0.17 ± 0.05^{b}
LPS (U/g prot)	347.12 ± 96.86^{b}	530.21 ± 182.86^{ab}	738.90 ± 103.62^{a}	595.25 ± 38.05^{a}	606.66 ± 5.76^{a}
TPS (U/mg prot)	1756.65 ± 692.00^{c}	5693.81 ± 500.69^{a}	3751.88 ± 319.65^{b}	3593.60 ± 749.13^{b}	3676.54 ± 792.99^{b}

Table 6: Serum antioxidant properties of *M. salmoides* at different stocking densities.

Parameter	D1	D2	D3	D4	D5
SOD (U/mL)	31.39 ± 1.77^{b}	45.78 ± 8.41^{a}	37.82 ± 5.44^{ab}	32.47 ± 1.90^{b}	33.81 ± 2.37^{b}
AKP (king unit/100 mL)	10.16 ± 0.73^{a}	6.67 ± 2.08^{b}	10.51 ± 0.36^{a}	6.51 ± 0.25^{b}	6.80 ± 0.48^{b}
CAT (U/mL)	14.84 ± 3.44^{b}	18.44 ± 4.80^{ab}	26.12 ± 0.21^{a}	16.82 ± 4.52^{b}	11.70 ± 2.61^{b}
MDA (nmol/mL)	36.15 ± 5.76^{a}	4.58 ± 1.87^{c}	6.56 ± 1.25^{bc}	7.69 ± 0.69^{bc}	13.03 ± 2.66^{b}
LZM (μg/mL)	19.97 ± 1.73^{c}	24.58 ± 1.16^{ab}	21.61 ± 0.56^{bc}	26.90 ± 3.19^{a}	28.63 ± 1.04^{a}

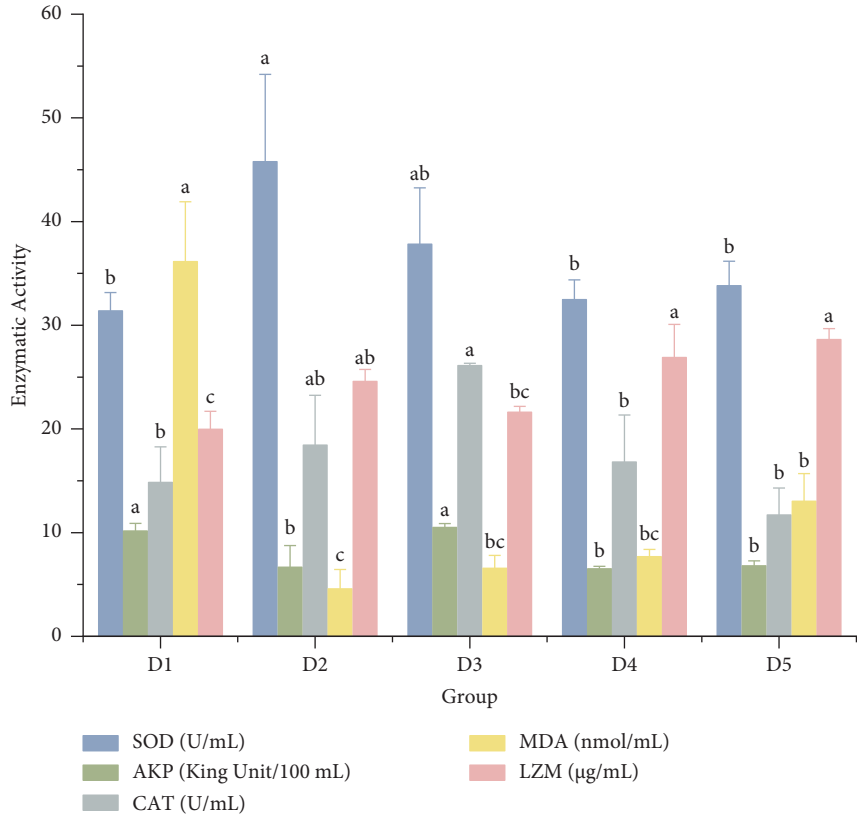

Figure 1: Serum antioxidant properties of *M. salmoides* at different stocking densities.

Table 7: Antioxidant properties in the livers of *M. salmoides* at different stocking densities.

Parameters	D1	D2	D3	D4	D5
SOD (U/mg prot)	129.89 ± 1.20^{bc}	120.42 ± 0.81^{c}	134.91 ± 5.16^{ab}	117.54 ± 0.30^{c}	142.71 ± 9.33^{a}
AKP (king unit/g prot)	94.13 ± 8.70^{b}	105.20 ± 7.85^{b}	100.55 ± 6.79^{b}	120.91 ± 20.16^{b}	182.48 ± 33.24^{a}
CAT (U/mg prot)	21.43 ± 6.45	15.94 ± 3.41	16.38 ± 4.49	17.22 ± 0.88	22.97 ± 2.42
MDA (nmol/mg prot)	0.16 ± 0.09^{b}	0.20 ± 0.08^{b}	0.30 ± 0.01^{ab}	0.30 ± 0.06^{ab}	0.44 ± 0.04^{a}
LZM (μg/mL)	1.38 ± 0.29^{b}	1.73 ± 0.01^{b}	2.35 ± 0.05^{a}	1.47 ± 0.22^{b}	2.76 ± 0.11^{a}

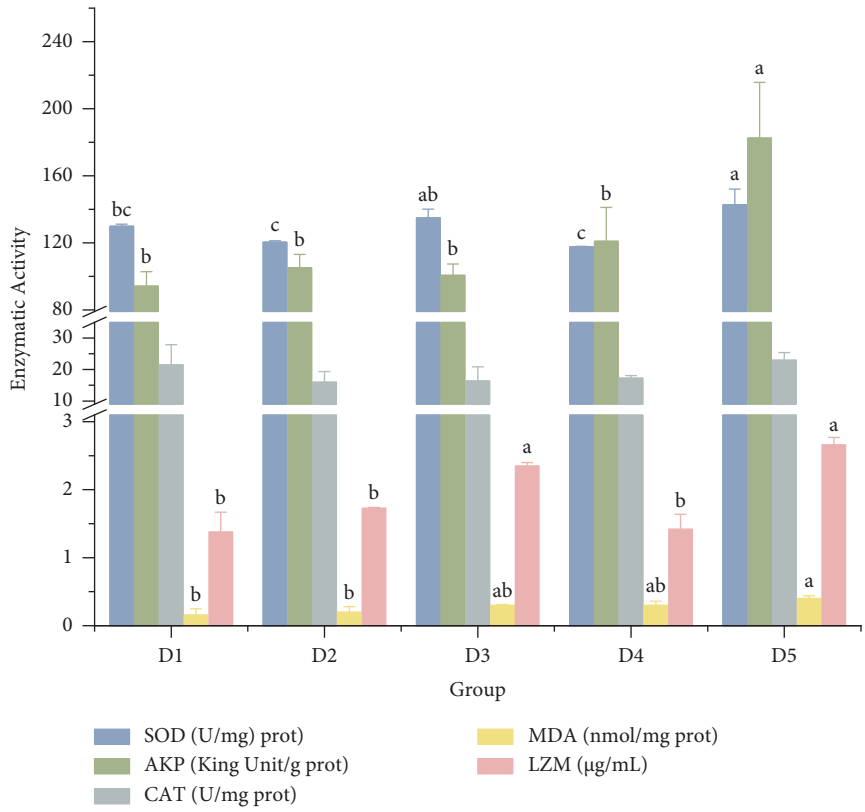

Figure 2: Antioxidant properties of the livers of *M. salmoides* at different stocking densities.

4. Discussion

Determining the optimal stocking density of fish at their specific age and size is a key factor for the success of aquaculture and plays a critical role regarding growth performance, stress parameters, and the health of cultured fish [21]. Therefore, it is important to identify the effects of stocking density on fish production, physiology, and immune functions. Stocking density does not markedly affect the survival rate of juvenile gilthead seabream (*Sparus aurata*) [22], which is consistent with the results of the present study, in which the survival rate of *M. salmoides* exceeded 90% at all stocking densities, with no significant differences among the groups. Growth performance and feed efficiency of cultured fish generally decrease with increasing stocking density [23–25]; however, growth performance may also improve or remain unaffected by increased stocking density within a certain range [26]. In the present study, the growth performance of *M. salmoides* decreased and then increased with the increase of the stocking density, and it was higher at the highest density (group D5) and the lowest density (group D1). The total incremental yield increased by 63.73%, 52.82%, 60.93%, and 45.08%, respectively, at a stocking density of group D5 (100 fish/m^2) compared with the other densities, and the feed conversion ratios were reduced by 5.65%, 12.03%, 26.42%, and 21.48%, respectively, compared with the other densities (group D1–D4). This may be because the overall size of *M. salmoides* was small in this test cycle, and the highest density tested here was insufficient to cause additional environmental stress. Thus, group D5 (100 fish/m^2) was a more suitable density condition for this culture stage, and growth performance and culture efficiency were improved. At the end of the culture trial, the average body weight of *M. salmoides* in each test group ranged from 110.44 to 130.13 g. In a previous study, we found that 65 fish/m^2 significantly improved the growth performance and culture efficiency of *M. salmoides* with an initial body mass of 109.00 ± 10.26 g after 63 days of culture [27]. We speculate that in the subsequent breeding after the end of this test period, the stocking density of group D5 (100 fish/m^2) may gradually cause higher pressure and inhibit the growth of fish. Therefore, the appropriate stocking density for the subsequent breeding price range and the differences in the physiological functions of the breeding objects at different densities remain to be determined.

Protein is the most important nutrient in muscle tissue-derived food, and fish muscle typically contains 11%–24% crude protein [28]; however, in extreme cases, this content may be <6% or >25% [29, 30]. In the present study, the crude protein content exceeded 19% in all groups, and it was significantly higher at stocking densities of groups D3 and D5 (80 and 100 fish/m^2).

The lowest crude ash content in the muscle of *M. salmoides* occurred at the lowest density (group D1), and the crude ash content of muscle increased with increasing stocking density, indicating that the muscle of cultured fish at lower stocking densities had less inorganic content and

was healthier, which is similar to that of juvenile macrohybrid sturgeon [31] and African catfish (*Clarias gariepinus*) [32]. The crude fat content in the livers of *M. salmoides* at the highest density (D5) was significantly lower than that in the low-density group, indicating that the livers of fish were healthier at higher stocking densities, probably because juveniles in the high-density group were subjected to the most pronounced chronic stress and thus required more energy to resist the high intensity of stress and food competition, resulting in lower liver fat content.

Amino acids mostly occur in protein-bound form, while there are fewer free amino acids. The flavor of fish meat is mainly determined by the amount of free amino acids, especially the content of amino acids conveying the fresh flavor. In the current study, the content of fresh amino acids in muscle tissue of *M. salmoides* in all groups accounted for about 50% of the total free amino acid content, indicating that the flavor of the fish in all groups was fresher and stocking density had no significant effect on the fresh flavor of the muscle. This is consistent with the results of a previous study on free amino acids in muscle tissue of Arctic salmon (*Salvelinus alpinus*) at different stocking densities [33]. Some free amino acids in fish also reflect the degree of microbial spoilage and are precursors of biogenic amines, among which histidine, lysine, ornithine, and glutamine may be sources of highly undesirable histamine, cadaverine, and putrescine in fish tissues, with a tendency to form higher levels of histamine at higher levels, thus reducing the taste and flavor of fish [34]. In the current study, the content of histamine (conveying bitterness) reached a threshold value in all experiments and tended to decrease with increasing stocking density, reaching a minimum at a stocking density of group D5 (100 fish/m^2), thus the muscle bitterness of *M. salmoides* was lower at this density than at other densities.

Changes in free amino acid concentrations in tissues also reflect the nutritional status of the organism [35], which has a crucial role in maintaining metabolism, growth, reproduction, and immune responses. Muscle, as the main tissue of the fish body, is the main storage site of free amino acids [36, 37]. In the present study, the muscle essential amino acid and total free amino acid levels of *M. salmoides* were higher at a stocking density of group D3 (80 fish/m^2), indicating that the fish muscle nutrition was superior at this density. This may be because the concentration of free amino acids depends on many factors such as species, dietary history, and the time and place of sampling after feeding [38], and various factors can cause instability in muscle free amino acid content.

Fatty acids (especially PUFA) play a crucial role in the growth and development of aquatic animals [39]. In the present experiment, 26 fatty acids were found in the muscle of *M. salmoides*. SFA are the main energy suppliers in the β-oxidation process of fish [40]. C16:0 and C18:0 are the main components of SFA [41]. In the current study, C16:0 and C18:0 content in muscle accounted for a relatively high proportion, which was consistent with the above results. PUFAs can not only regulate lipid metabolism and promote growth and development but also effectively improve human immunity. We observed no effect of stocking density on PUFA content. The balanced composition of human essential fatty acids, i.e. linoleic acid (C18:2n-6) and linolenic acid (C18:3n-3, C18:3n-6), is essential for human health and plays a very important role in growth, development, and physiological activities; thus, their content is an important index for the evaluation of fish muscle nutrition. In this study, stocking density had no significant effect on the content of essential fatty acids in the muscle of *M. salmoides*. DHA and EPA are n-3 long-chain highly unsaturated fatty acids which can be synthesized from ingested linolenic acid, and they are also important indicators for the evaluation of the nutritional value of fish muscle. Unsaturated fatty acids can lower blood lipids, inhibit platelet agglutination, lower blood pressure, improve blood flow, and exert antitumor, antiinflammatory, and immunomodulation effects, which can prevent human cardiovascular diseases and contribute to human brain development [42]. In the present study, stocking density had no significant effect on DHA and EPA. The n-3/n-6 ratio is a key indicator of the nutritional value of fatty acids, with higher n-3/n-6 ratios indicating higher nutritional value [43]. The FAO/WHO recommends an n-3/n-6 ratio in the daily diet of at least 0.1 to 0.2 [44]. In the present study, the ratios of muscle n-3/n-6 of *M. salmoides* in each test group ranged from 0.64 to 1.25, thus exceeding 0.2, indicating that *M. salmoides* cultured under land-based round pond recirculating water conditions had high nutritional value. Overall, the fatty acid composition of muscle tissue in the present study was apparently not affected by stocking density, which was previously also shown to have no significant effect on the fatty acid content of gilthead seabream [22] and Siberian sturgeon (*Acipenser baerii*) [45].

Digestive enzymes are important compounds mediating nutritional processes, and their activity represents the ability of the organism to digest and absorb nutrients [46]. Amylase, LPS, and TPS are important digestive enzymes for aquatic animals and affect their growth performance by influencing nutrient absorption [47]. Increasing stocking densities lead to a pronounced decrease in digestive enzyme activity in Nile tilapia (*Oreochromis niloticus*) [48], Chinese penaeid shrimp (*Palaemonetes sinensis*) [49], and sarana (*Puntius sarana*) ([21]. This is similar to the results of the present study, where AMS activity in the intestine of *M. salmoides* gradually decreased with increasing stocking density, and LPS and TPS activities showed a trend of first increasing and then decreasing. In general, the culture conditions of intermediate density (group D2-D3) under the conditions of land-based circular-pond circulating water were beneficial to improving the activity of digestive enzymes of *M. salmoides*, which was beneficial to the digestion and absorption of food nutrients.

Reactive oxygen species scavenge excess superoxide produced in the body due to stressful environments such as increased stocking densities, which can cause oxidative stress when the antioxidant defense system is unable to neutralize these compounds [50]. These antioxidant enzymes of the animal defense system are used as indicators of oxidative stress [51–53], as organisms avoid or repair tissue damage through enzymatic antioxidant defense protection systems (such as SOD, AKP, CAT, and MDA) to maintain

reactive oxygen species homeostasis. In *M. salmoides* serum, SOD, AKP, and CAT activities were highest at a stocking density of group D3 (80 fish/m^2), MDA gradually increased with increasing stocking density, and LZM activity reached its maximum at a stocking density of group D5 (100 fish/m^2). In the liver, SOD, AKP, CAT, and LZM activities showed an overall increasing trend with increasing stocking density and reached the maximum at a stocking density of group D5 (100 fish/m^2). A similar phenomenon was found in gilthead seabream [54] and common carp (*Cyprinus carpio* L.) [55, 56]. As the level of antioxidant enzymes in serum fluctuates and the liver exerts a storage function, liver enzyme activity is more representative of the antioxidant capacity of the organism than blood. Our results showed that a stocking density of group D5 (100 fish/m^2) was conducive to improving the overall antioxidant capacity of *M. salmoides*.

5. Conclusion

A good balance between growth and physiological health of cultured fish and economic yield benefits is key to successful aquaculture practice. In this study, although the crude protein, essential amino acid, and total free amino acid levels were the highest in group D3, group D5 showed markedly improved growth performance, total incremental yield, and reduced feed energy consumption in *M. salmoides*, with the greatest culture efficiency. Furthermore, this stocking density significantly improved the antioxidant capacity of the fish, which was beneficial to its health. In conclusion, the stocking density of 100 fish/m^2 is appropriate for culturing *M. salmoides* at a size stage of 50.83–130.13 g in land-based round pond circulating water conditions.

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 they have no conflicts of interest.

Authors' Contributions

Guangping Cheng and Yanqun Ma conceived the study. Nan Si, Baifei Jie, Lanfang Dong, Changhui Gu, Man Cheng, Yunyong Wei, and Kai Yu designed the study and conducted the research. Data analysis, interpretation, and writing of the manuscript were performed by Nan Si.

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