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Assessment of zootechnical parameters, intestinal digestive enzymes, haemato-immune responses, and hepatic antioxidant status of *Pangasianodon hypophthalmus* fingerlings reared under different stocking densities

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Abstract
A 3-month experiment was designed to investigate the effects of different stocking densities on growth, stress markers, intestinal digestive enzymes, hepatic antioxidant biomarkers, serum immunity, and survivability of striped catfish, *Pangasianodon hypophthalmus*. The relationship between fish stocking densities and water quality parameters was also assessed. Healthy fish ($n = 1260$ individuals, 17.52 ± 0.20 g) were raised in twelve fiberglass tanks. Triplicate fish groups with stocking densities of 60, 90, 120, or 150 fish per 1 m^3 were designed, further referred to as SD60, SD90, SD120, and SD150 groups. Fish feeding was done using a commercially purchased well-balanced diet three times per day with a feeding rate of 3% of the wet fish weight. Results revealed that un-ionized ammonia, nitrite, and pH were significantly elevated, and dissolved oxygen levels were decreased significantly with increasing the stocking densities. The survival rates and growth significantly decreased with increasing fish stocking densities. The intestinal lipase, protease, trypsin, and amylase enzymes decreased significantly ($P < 0.05$) along with increased stocking densities. Moreover, significant decreases were noticed in total protein, lysozyme activity, and globulin levels when the stocking density was higher than 60 fish/m^3. Conversely, serum stress biomarkers (such as blood glucose and cortisol), transaminases, alkaline phosphatase, and blood urea nitrogen were significantly elevated with increasing stocking densities. Hepatic CAT, SOD, and T-AOC were decreased; meanwhile, hepatic MDA levels were significantly increased, together with the stocking rates. In the end, we found that the SD150 group recorded the lowest growth rates, immune responses, and antioxidant capacity and the highest stress markers as blood glucose and cortisol. In this context, we can conclude that the stocking 60 fish/m^3 resulted in better growth, survival, immunity, antioxidant status, and overall performances of striped catfish.

Keywords Stocking density · Antioxidants · Stress biomarkers · Immunity · Water quality

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Introduction

In recent years, aquaculture has grown greatly to supply humans with nutritious protein sources (FAO 2018; Maulu et al. 2021). Globally, fish farming is a common profitable occupation for many farmers (Mansour et al. 2017; Khalil et al. 2021). Nevertheless, it faces challenges that may negatively influence productivity and reduce profits and economic returns (Mansour et al. 2021; El-Ouny et al. 2023). Because of the continuously elevated fish requirement as human food, the aquaculturists and fish farmers focused on the intensive farming system to enlarge their production (Kord et al. 2021; Adam et al. 2023). However, this farming system may accelerate the emergence of many fish disease-causing microorganisms and raise the incidence of serious outbreaks among the farmed fish (Abdel-Latif and Khafaga 2020). Moreover, intensive systems face other problems, such as higher operational costs, deteriorated water quality, increased land requirements for feed production, effluent discharges, excessive water usage, and several others (Khalil et al. 2022a; Kord et al. 2022).

Optimizing fish stocking densities in culture facilities is essential for an intensive culture system because it directly affects fish growth rates and survival percentages (Nageswari et al. 2022). For this reason, the density of fish stocked in farm facilities will affect the yield and farm profitability (Chowdhury et al. 2020). In general, fish farming with high densities significantly altered the growth, intestinal histomorphology, and muscle quality of *Ictalurus punctatus* fingerlings (Refaey et al. 2018). Higher densities also depressed the growth of *Oncorhynchus mykiss* (Zahedi et al. 2019). Moreover, high densities negatively influenced the growth and antioxidant activities of *Carassius gibelio* (Onxayvieng et al. 2021). Alternatively, low stocking densities result in a low yield per unit surface area or water volume and are economically less attractive to farmers (Chowdhury et al. 2020).

Striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878), is a commonly cultured omnivorous fish species in several Asian countries and has been recently presented as an exotic species in several countries, such as Egypt (Abd-elaziz et al. 2023). It can feed on algae, zooplankton, natural fauna, and higher plants and accept manufactured feed (Allam et al. 2020; Abdel-Latif et al. 2022, 2023a, 2023b). It has excellent growth and, as an air breather, is easy to farm, showing a high tolerance to stressors and increased demand for food (Ali et al. 2013; Adam et al. 2023). The influences of high stocking densities on the growth and physiological responses of *P. hypophthalmus* have been assessed in several studies. It was found that larval *P. hypophthalmus* raised at high densities had lowered growth rates, survival percent, and yield (Slembrouck et al. 2009). Likewise, increased stocking rates deteriorated the rearing water quality and decreased the survival and growth of *P. hypophthalmus* fingerlings raised in cement tanks (Malik et al. 2014). Moreover, the growth and yield were significantly decreased in cage-farmed *P. hypophthalmus* reared under high-density conditions (Chowdhury et al. 2020). Nageswari et al. (2022) declared that a stocking density of 270 individuals/1 m^3 negatively influenced the survival, growth, digestive enzymes, and metabolic enzymes in *P. hypophthalmus* fingerlings reared in a biofloc-based system. However, no research has yet assessed the effects of different stocking rates of *P. hypophthalmus* fingerlings cultivated in tanks on welfare and health parameters. The foremost purpose of the present study is to evaluate the water quality, growth performance, somatic organ indices, body composition, digestive enzyme, haemato-immune parameters, serum stress biomarkers, and hepatic antioxidants of *P. hypophthalmus* fingerlings raised at several stocking densities.

Material and methods

Fish collection and acclimatization

Healthy *P. hypophthalmus* fingerlings ($n = 1260$), with a mean initial weight of 17.52 ± 0.20 g, were procured from a private local fish hatchery at Borg El Arab region, Alexandria (Egypt). These fish were kept indoors to be acclimated and conditioned in twelve plastic cube tanks (1000 L) for 2 weeks. Before receiving the experimental fish, the tanks were thoroughly disinfected, left overnight, washed with clean water, and left to dry in the sun for 2 days. These tanks were filled with de-chlorinated tap water. During the conditioning and experiment, fish were fed a well-balanced 30% crude protein diet (Table 1) that covered all the fish requirements (NRC 2011).

Table 1 Ingredients and proximate chemical composition (% on dry matter basis) of the basal diet used in the current study

Feed ingredients	%
Fish meal (62% CP)	10.0
Soybean meal (48% CP)	34.0
Corn gluten meal (60% CP)	3.5
Rice bran	14.0
Yellow corn meal	15.0
Wheat bran	9.0
Wheat flour	13.0
Sunflower oil	0.70
Vitamin and mineral premix[a]	0.30
Dicalcium phosphate	0.50
Total	100
Proximate chemical analysis (%) on DM basis	
Dry matter (DM)	90.19
Crude protein (CP)	30.82
Ether extract (EE)	6.93
Ash	6.59
Crude fiber (CF)	8.81
Nitrogen-free extract (NFE)[b]	46.85
Gross energy (GE; KJ/g diet DM)[c]	18.06
Protein to energy ratio (P/E) ratio[d]	17.06

[a]Composition of vitamin and mineral premix mixture (per kg premix): vitamins such as vitamin B1 (700 mg), vitamin C (500 mg), vitamin B2 (3500 mg), vitamin B6 (1000 mg), vitamin B12 (7 mg), vitamin A (8,000,000 IU), vitamin D3 (2,000,000 IU), vitamin E (7000 mg), vitamin K3 (1500 mg), biotin (50 mg), folic acid (700 mg), nicotinic acid (20,000 mg), and pantothenic acid (7000 mg). Minerals such as zinc (40 g), iron (20 g), copper (2.7 g), iodine (0.34 g), manganese (53 g), selenium (70 mg), cobalt (70 mg) and calcium carbonate as carrier up to 1 kg

[b]NFE = 100—(CP + EE + CF + ash)

[c]GE was calculated based on 23.60, 39.40, and 17.20 kJ/g of CP, EE and NFE

[d]P/E ratio was calculated as mg crude protein/KJ GE

Fish stocking, rearing, and experimental design

After conditioning, fish were allocated into 12 indoor circular fiberglass tanks, each tank holding 1000 L water. Fish were stocked in these tanks in triplicate at four stocking densities (SD): 60, 90, 120, and 150 fish/m^3, further referred to as SD60, SD90, SD120, and SD150 groups, respectively. The lighting schedule was fit at 12 h:12 h light and dark cycle. All rearing tanks were well-aerated. Fish were hand-fed three times daily at a rate of 3% of the body weight of fish, changed every 2 weeks along with the fish's growth. Fish were reared for 90 days. Every 2 days, 30% of the water in each tank was substituted with well-aerated water, and the excreta were siphoned off. After the termination of feeding (90 days), the final fish weight (FW) was measured by splitting the total weight of nine fish from each treatment. Moreover, the fish number in each tank was totaled to verify the survival percentage.

Measurement of growth parameter, survival, and organo-somatic indices

$$\text{Weight gain}\frac{(\text{WG})\text{g}}{\text{fish}} = \text{Final weight (Wt90)} - \text{Initial weight (Wt0)}$$

$$\text{WG \%} = 100 \times (\text{Wt90} - \text{Wt0}) / \text{Wt0}$$

$$\text{Average daily gain(g / fish/day)} = (\text{Wt90} - \text{Wt0})/ 90$$

$$\text{Specific growth rate (\%/day)} = [\text{Ln Wt90} - \text{Ln Wt0}] \times 100 / 90$$

$$\text{Feed intake (FI)} = \text{Total quantity of feed eaten by fish after 90 days of the rearing experiment}$$

$$\text{Feed conversion ratio} = \text{FI (g)} / \text{WG (g)}$$

$$\text{Protein efficiency ratio} = \text{WG (g)} / \text{protein intake in feed (g)}$$

$$\text{Protein productive value (\%)} = 100 \times [\text{protein gain in fish (g)/protein intake in feed (g)}]$$

$$\text{Energy utilization (\%)} = 100 \times (\text{ET} - \text{EI})/\text{energy intake (kJ)}$$

ET is the energy in the fish carcass after 90 days of the experiment, and EI is the energy in the fish carcass at the start of the experiment.

Survival percent (%) = 100 × (Fish number after the end / Fish number at the beginning).

The organ somatic indices were calculated (3 fish /tank) as g per 100 g body weight as follows:

$$\text{Viscera somatic index (\%)} = [\text{Weight of the viscera (g)} / \text{Fish weight (g)}] \times 100$$

$$\text{Kidney somatic index (\%)} = [\text{Weight of the kidney (g)} / \text{Fish weight (g)}] \times 100$$

$$\text{Hepatosomatic index (\%)} = [\text{Weight of the liver (g)} / \text{Fish weight (g)}] \times 100$$

$$\text{Spleen somatic index (\%)} = [\text{Weight of the spleen (g)} / \text{Fish weight (g)}] \times 100$$

Water quality parameters

Water samples were taken every 10 days from each tank of the experimental groups before water renewal to examine the water parameters. Water temperature (°C), dissolved oxygen (DO; mg/L), and pH values were assessed by using a water thermometer and HI9829 multiparameter HANNA apparatus (Nasr City, Egypt). Nitrite (NO_2; mg/L) and unionized ammonia (NH_3; mg/L) were evaluated by using a portable spectrophotometer 2000 (HACH Co., USA).

Assessment of the whole-body chemical composition

Three fish per tank ($n=9$ fish per group) were euthanized using an overdose of clove oil (450 mg/L). Fish samples were frozen at − 20 °C to analyze the whole-body chemical composition. The crude protein (CP; %), moisture (%), crude lipid (CL; %), and ash (%) were evaluated in line with the guidelines and procedures described by AOAC (2012). A detailed description of these methodologies has been formerly published (Abdel-Latif et al. 2023b).

Sampling procedures

After the termination of the feeding, the fish were not fed for 1 day before sampling. Fish were anesthetized by using clove oil (50 µL/L). Blood sampling was done for serum collection. Nine fish were sampled for organs and tissue samples for further analysis. Nine fish per group were collected, and blood samples were withdrawn from the caudal vessels without using anticoagulants. The collected blood samples were left in plastic tube racks for 3 h at room temperature. Serum samples were collected after centrifugation at 3000 rpm for 10 min at 4 °C. The collected sera were then refrigerated at − 20 °C until further biochemical measurements (serum immunity and stress biomarkers). After blood sampling, the liver, viscera, spleen, and kidney were collected and weighed individually. These specimens were used for the calculation of organ somatic indices. Liver and intestinal samples (9 per group) were collected on ice. Homogenized samples were centrifuged at 5000 rpm for 10 min at 4 °C. The sediment was discarded, and the supernatant was saved in sterile tubes and then cooled at − 20 °C.

Serum biochemical, immunity, and stress biomarkers

Total protein (TP), albumin (ALB), blood urea nitrogen (BUN), glucose, cortisol, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were determined using specific diagnostic kits (Biodiagnostic Co., Egypt). TP and ALB values were assessed (Doumas et al. 1981). Serum globulin (GLO) values were evaluated by subtracting ALB values from TP values. BUN and cortisol levels were measured according to the methods explained in (Coulombe and Favreau 1963; Foo and Lam 1993). Liver enzymes AST, ALT, and ALP were also assessed (Reitman and Frankel 1957; Belfield and Goldberg 1971). Serum lysozyme (LYZ) activity was assessed by a turbidimetric assay using a suspension of *Micrococcus lysodeikticus* (EC 3.2.1.17) (Sigma, USA) (Ellis 1990). In summary, this bacterial isolate was used as a substrate. Serum samples were added to the bacterial suspension in a microtiter plate. The absorbance reduction was assessed at 480 nm after incubation for 0.5 and 4.5 min at 23 °C. Results were taken by a microplate reader. Serum LYZ activities were calculated from a standard curve prepared from chicken egg white lysozyme (Sigma,

USA). BUN levels were determined by a urease-established enzymatic conductivity method, according to Horak and Sunderman (1972). The cortisol levels (ng/mL) were analyzed by the ELISA method using a Neogen Corporation ELISA kit (USA), according to Inoue et al. (2008).

Hepatic oxidative stress biomarkers

The oxidative stress biomarkers were assayed in the liver homogenate samples using commercially acquired diagnostic kits (MyBioSource Inc., USA) according to the recommendations supported by the manufacturer. Hepatic catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (T-AOC) were determined according to the methods described by Aebi (1984), McCord and Fridovich (1969), Paglia and Valentine (1967), and Amado et al. (2009), respectively. Hepatic malondialdehyde (MDA) levels were assessed at 532 nm by the thiobarbituric acid method in line with the steps described by Uchiyama and Mihara (1978) and Ohkawa et al. (1979).

Intestinal digestive enzymes

The intestinal enzymes (U/mg protein) were assayed in the intestinal samples by commercially purchased diagnostic kits (Cusabio Biotech Co. Ltd., China) in accordance with the regulations provided by the suppliers. The intestinal amylase, lipase, protease, and trypsin activities were examined by the methods depicted by Bernfeld (1955), Shihabi and Bishop (1971), Khantaphant and Benjakul (2008), and Erlanger et al. (1961), respectively.

Statistical analysis

Results were investigated by one-way ANOVA and were expressed as means ± SE. $P < 0.05$ was used to determine the significant differences between test groups. Polynomial regression was performed to assess the effect of stocking density on FW, FI, WG%, and SGR. The analyses were performed using the SPSS program (version 17 SPSS Inc. USA).

Results

Water quality

The changes in the measured parameters of the water in the rearing tanks contained fish at different densities (every 10 days) in all groups throughout the 90 days of the experiment are illustrated in Figs. 1 and 2. The average range of water temperature throughout the study was 29.0–32.0 °C, with no significant changes ($P > 0.05$) among the fish groups (Fig. 1). However, the mean DO, pH, NH_3, and NO_2 levels differed significantly between the groups. DO levels were significantly decreased alongside the increase in the stocking densities ($P < 0.05$), and the lowest DO levels were recorded in the SD150 group (5.40 mg/L) (Fig. 1). Conversely, the pH, NH_3, and NO_2 levels in the rearing water were significantly elevated together with the stocking density rates, and their highest values were noted in the SD150 group. The lowest values were found in the SD60 group (Fig. 2).

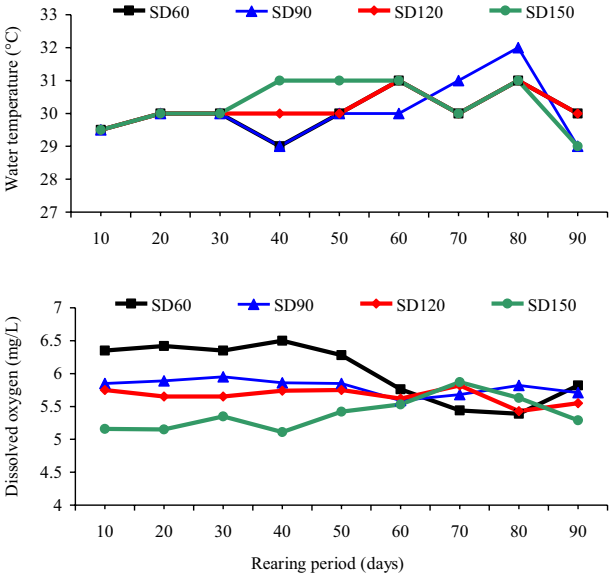

Fig. 1 Changes in the water temperature (°C) and dissolved oxygen (mg/L) levels in the water of the tanks stocked with *P. hypophthalmus* and reared in different stocking densities (SD) for 90 days

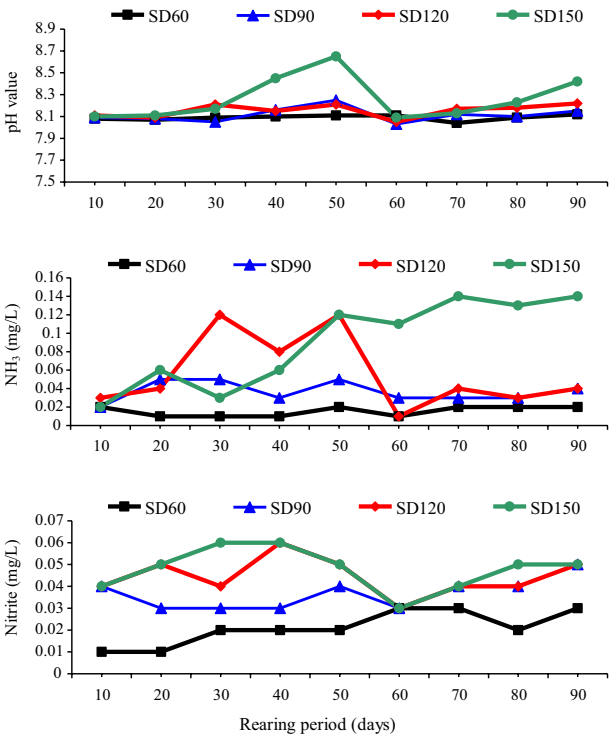

Fig. 2 Changes in pH values, unionized ammonia (NH3; mg/L), and nitrite (mg/L) levels in the water of the tanks stocked with *P. hypophthalmus* and reared in different stocking densities (SD) for 90 days

Growth, survival, and feed utilization

The growth performance, feed utilization, and survival of *P. hypophthalmus* fingerlings raised under different densities are represented in Table 2. The initial fish weights were not statistically ($P > 0.05$) varied among the groups. The mean values of FW, WG, ADG, WG%, SGR, and SP% were significantly decreased ($P < 0.05$) together with the stocking density rates of fish in the rearing tanks. The highest values of these parameters were noticed in the SD60 group (60 fish/m^3), followed by the SD90 group (90 fish/m^3) and the SD120 group (120 fish/m^3). In comparison, their lowest levels were noted in the SD150 group (150 fish/m^3). Of interest, no fish have died in the SD60 group with a 100% SP%. A similar trend was noticed in the PER, PPV, and EU values of fish raised in different groups ($P < 0.05$; Table 2). Differently, FI and FCR increased significantly as the stocking densities increased ($P < 0.05$; Table 2). Significantly lower FI and FCR values were recorded in the SD60 group (60 fish/m^3). The relationship among FW, WG%, SGR, and FI and the different fish stocking densities are presented in Fig. 3. It was shown that the optimum density of *P. hypophthalmus* fingerlings was in the SD60 group (60 fish/m^3) with maximum FW, WG%, SGR, and lowest FI values among the experimental groups.

Biometric indices and body composition

The organ somatic indexes and body composition analysis of *P. hypophthalmus* fingerlings raised under different densities for 90 days are presented in Tables 3 and 4, respectively. There were significant variations in VSI, KSI, HSI, and SSI between all experimental groups (Table 3), whereas the lowest values were noted in the fish group with the highest stocking density (SD150), and their highest values were recorded in the SD90 group. Alternatively, the highest moisture and ash % were found in the SD150 group (Table 4). Moreover, the lowest CP, EE, and carcass energy contents were noted in the SD150 group with the highest densities.

Serum immune and stress indices

The blood proteins (TP, ALB, and GLO values), liver function enzyme activities (ALT, AST, and ALP), and other serum biochemical indices (glucose, cortisol, BUN, and serum LYZ activity) of *P. hypophthalmus* fingerlings raised under different stocking densities are represented in Table 5. Significantly higher ($P < 0.05$) serum TP, ALB, and lysozyme activity were noticed in the SD60 group (60 fish/m^3), and their values were the lowest in the SD150 group (150 fish/m^3). Moreover, glucose, cortisol, BUN, ALT, AST, and ALP levels were increased significantly alongside the increase in fish stocking rates ($P < 0.05$; Table 5), and their highest levels were noted in the SD150 group.

Hepatic oxidative stress parameters

The values of hepatic enzymes (as CAT, SOD, and GPx), hepatic MDA, and T-AOC of *P. hypophthalmus* raised under different densities are depicted in Table 6. Hepatic CAT, SOD, and T-AOC levels were significantly lowered with increased fish stocking

Table 2 Effects of different stocking densities of *P. hypophthalmus* on the growth parameters, survival rates, and feed utilization indices after a rearing period of 90 days

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	SD90 (90 fish/m^3)	SD120 (120 fish/m^3)	SD150 (150 fish/m^3)	
Initial body weight (IBW; g/fish)	17.50 ± 0.02	17.60 ± 0.02	17.30 ± 0.02	17.70 ± 0.08	0.952
Final body weight (FBW; g/fish)	181.00 ± 0.33 a	143.50 ± 0.28 b	128.60 ± 0.12 c	105.50 ± 0.49 d	< 0.001
Weight gain (WG; g/fish)	163.50 ± 0.34 a	126.00 ± 0.28 b	111.10 ± 0.12 c	88.00 ± 0.50 d	< 0.001
Average daily gain (ADG; g/fish/day)	1.82 ± 0.03 a	1.40 ± 0.03 b	1.23 ± 0.01 c	0.98 ± 0.05 d	< 0.001
Weight gain percentage (WG%)	934.20 ± 1.91 a	719.90 ± 1.63 b	634.80 ± 0.69 c	502.78 ± 2.84 d	< 0.001
Specific growth rate (SGR; %/day)	2.59 ± 0.02 a	2.338 ± 0.02 b	2.216 ± 0.01 c	1.99 ± 0.05 d	< 0.001
Survival rate (SR; %)	100.0 ± 0.00 a	98.10 ± 1.85 a	95.80 ± 2.41 ab	90.00 ± 1.92 b	0.021
Total feed intake (FI; g/fish)	166.90 ± 0.66 d	175.20 ± 0.68 c	195.10 ± 0.45 b	227.20 ± 0.84 a	< 0.001
Feed conversion ratio (FCR)	1.02 ± 0.05 d	1.39 ± 0.03 c	1.76 ± 0.05 b	2.58 ± 0.05 a	< 0.001
Protein efficiency ratio (PER)	3.11 ± 0.09 a	2.28 ± 0.01 b	1.81 ± 0.01 c	1.23 ± 0.01 d	< 0.001
Protein productive value (PPV; %)	61.17 ± 0.13 a	41.02 ± 0.18 b	29.24 ± 0.22 c	19.07 ± 0.25 d	< 0.001
Energy utilization (EU; %)	31.98 ± 0.01 a	23.42 ± 0.12 b	16.77 ± 0.04 c	10.35 ± 0.25 d	< 0.001

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Duncan's multiple range test), $n = 9$

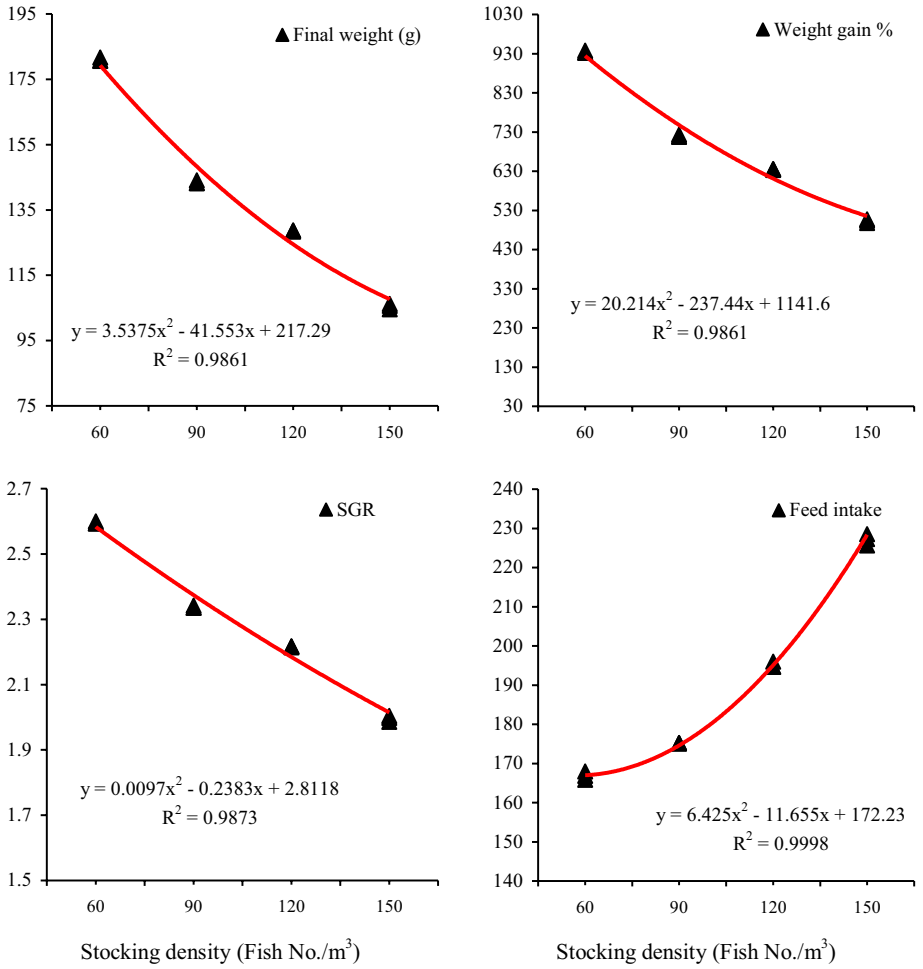

Fig. 3 The relationship between final body weight (FBW; g), specific growth rate (SGR; %/day), weight gain % (WG%), and feed intake (FI; g feed/fish) in different stocking densities of *P. hypophthalmus* and reared for 90 days

densities. On the contrary, MDA and GPx levels have an inverse trend. Interestingly, the highest CAT, SOD, and T-AOC levels were noticed in the SD60 group; meanwhile, the lowest MDA and GPx levels were noted in the SD60 group (Table 6).

Intestinal digestive enzymes

The intestinal enzymes of *P. hypophthalmus* raised under different stocking densities are shown in Table 7. The lipase, amylase, trypsin, and protease were the lowest in the SD150 group compared with other groups. However, the SD60 group exhibited the highest intestinal enzyme values (Table 7).

Table 3 Effects of different stocking densities on body somatic indices of *P. hypophthalmus* after a rearing period of 90 days

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	**SD90** (90 fish/m^3)	**SD120** (120 fish/m^3)	**SD150** (150 fish/m^3)	
Visceral somatic index (VSI; %)	23.20 ± 0.07 ab	23.6 ± 0.14 a	22.20 ± 0.21 b	20.90 ± 0.39 c	< 0.001
Kidney index (KI; %)	0.52 ± 0.06 b	0.63 ± 0.08 a	0.43 ± 0.02 c	0.44 ± 0.03 c	< 0.001
Hepatosomatic index (HSI; %)	2.29 ± 0.02 b	2.37 ± 0.08 a	2.24 ± 0.08 b	2.12 ± 0.01 c	< 0.001
Spleen index (SI; %)	0.35 ± 0.01 a	0.41 ± 0.02 a	0.26 ± 0.03 b	0.21 ± 0.01 b	< 0.001

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Tukey's HSD test), $n = 9$

Table 4 Effects of different stocking densities on the whole-body proximate composition (on wet weight basis) of *P. hypophthalmus* after a rearing period of 90 days ($n=3$; means ± S.E.M.)

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	**SD90** (90 fish/m^3)	**SD120** (120 fish/m^3)	**SD150** (150 fish/m^3)	
Moisture (%)	75.10 ± 0.22 b	75.40 ± 0.60 b	75.80 ± 0.48 ab	76.80 ± 0.24 a	0.033
Crude protein (CP; %)	17.70 ± 0.38 a	17.10 ± 0.64 ab	15.80 ± 0.26 bc	15.20 ± 0.27 c	0.009
Ether extract (EE; %)	4.80 ± 0.21 a	4.60 ± 0.24 a	4.50 ± 0.09 a	3.80 ± 0.19 b	0.033
Ash (%)	2.30 ± 0.09 c	2.90 ± 0.28 b	3.50 ± 0.09 ab	3.80 ± 0.25 a	0.003
Carcass energy content (Kcal/100 g)	552.66 ± 0.51 c	558.44 ± 0.74 a	553.47 ± 0.74 b	542.61 ± 0.21 d	< 0.001

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Duncan's multiple range test)

Table 5 Effects of different stocking densities on serum biochemical indices of *P. hypophthalmus* after a rearing period of 90 days

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	**SD90** (90 fish/m^3)	**SD120** (120 fish/m^3)	**SD150** (150 fish/m^3)	
Total protein (TP; g/dL)	10.37 ± 0.45 a	9.39 ± 0.41 a	7.47 ± 0.40 b	5.39 ± 0.36 c	< 0.001
Albumin (ALB; g/dL)	2.09 ± 0.10 c	2.19 ± 0.08 bc	2.39 ± 0.04 ab	2.47 ± 0.04 a	0.024
Globulin (GLO; g/dL)	8.28 ± 0.34 a	7.20 ± 0.43 a	5.09 ± 0.36 b	2.92 ± 0.39 c	< 0.001
Glucose (mg/dL)	67.00 ± 1.76 d	72.00 ± 1.07 c	81.10 ± 1.08 b	92.00 ± 0.69 a	< 0.001
Cortisol (ng/mL)	612.90 ± 9.76 d	637.00 ± 4.39 c	704.50 ± 5.53 b	736.00 ± 5.78 a	< 0.001
Lysozyme (ng/mL)	41.20 ± 0.67 a	38.70 ± 1.24 a	32.90 ± 0.79 b	27.20 ± 0.88 c	< 0.001
Aspartate transaminase (AST; IU/L)	11.30 ± 0.41 c	13.10 ± 0.25 c	15.60 ± 0.47 b	18.50 ± 1.07 a	< 0.001
Alanine transaminase (ALT; IU/L)	13.70 ± 0.57 c	14.10 ± 0.12 c	16.80 ± 0.38 b	20.50 ± 0.49 a	< 0.001
Alkaline phosphatase enzyme (ALP; IU/L)	81.80 ± 4.21 d	93.10 ± 3.44 c	110.50 ± 2.72 b	121.30 ± 1.78 a	< 0.001
Blood urea nitrogen (BUN; mg/dL)	5.90 ± 0.29 c	6.35 ± 0.07 bc	6.56 ± 0.06 b	7.16 ± 0.06 a	0.004

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Duncan's multiple range test), $n = 9$

Table 6 Effects of different stocking densities on hepatic antioxidant parameters of *P. hypophthalmus* after a rearing period of 90 days

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	**SD90** (90 fish/m^3)	**SD120** (120 fish/m^3)	**SD150** (150 fish/m^3)	
Catalase (CAT) (U/mg protein)	4.62 ± 0.20 a	4.04 ± 0.13 b	3.70 ± 0.13 b	2.71 ± 0.04 c	< 0.001
Malondialdehyde (MDA; nmol/mg protein)	0.23 ± 0.01 c	0.28 ± 0.01 bc	0.33 ± 0.01 b	0.45 ± 0.02 a	< 0.001
Glutathione peroxidase (GPx; U/mg protein)	4.73 ± 0.18 c	5.22 ± 0.12 b	5.48 ± 0.12 b	6.21 ± 0.14 a	< 0.001
Superoxide dismutase (U/mg protein)	5.94 ± 0.31 a	5.34 ± 0.07 b	4.82 ± 0.08 b	3.74 ± 0.18 c	< 0.001
Total antioxidant capacity (T-AOC; U/mg protein)	0.50 ± 0.05 a	0.41 ± 0.01 ab	0.36 ± 0.02 bc	0.27 ± 0.01 c	0.002

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Duncan's multiple range test), $n = 9$

Table 7 Effects of different stocking densities on intestinal digestive enzymes activities of *P. hypophthalmus* after a rearing period of 90 days

Parameters	Stocking density (number of fish/ m^3)				*P* value
	SD60 (60 fish/m^3)	**SD90** (90 fish/m^3)	**SD120** (120 fish/m^3)	**SD150** (150 fish/m^3)	
Lipase (U/mg protein)	56.10 ± 0.75 a	52.10 ± 0.95 ab	48.60 ± 1.11 b	40.30 ± 3.79 c	0.004
Amylase (U/mg protein)	0.58 ± 0.03 a	0.56 ± 0.01 ab	0.49 ± 0.01 bc	0.42 ± 0.02 c	0.006
Protease (U/mg protein)	24.90 ± 0.63 a	21.80 ± 0.77 b	19.60 ± 0.45 c	16.50 ± 0.38 c	< 0.001
Trypsin (U/mg protein)	282.80 ± 3.46 a	263.10 ± 3.18 b	238.10 ± 2.87 c	218.40 ± 3.73 d	< 0.001

Data was expressed as means ± S.E.M

Means having different letters in the same row are significantly different at $P < 0.05$ (Duncan's multiple range test), $n = 9$

Discussion

Although a high stocking density (HSD) will positively impact farm productivity from higher total biomass produced, caution is required because, in high-density cultures, infectious pathogens can rapidly spread (Abdel-Latif et al. 2020a). On the other hand, a low stocking density will reduce the yield per culture unit and income (Chowdhury et al. 2020). Therefore, this study examined the influences of different stocking densities of *P. hypophthalmus* fingerlings raised in circular fiberglass tanks on fish's overall performance, health, and welfare.

HSD rates of *P. hypophthalmus* fingerlings caused a significant decline in mean values of DO and an increase in mean values of pH, NH_3, and NO_2. These findings were similar to other stocking density studies. For instance, it was noted that DO levels were decreased, and NH_3, NO_2, and phosphates levels were increased in the tanks of two important carp species known as *Catla catla* and *Labeo rohita* reared at high density (Sharma and Chakrabarti 2003). Also, the total ammonia nitrogen (TAN) and NO_2 levels were elevated, and DO levels were decreased alongside the increase of the rearing density of *Oreochromis niloticus*×*O. aureus* hybrids (Al-Harbi and Siddiqui 2000). In a similar trend, DO concentrations dropped, and NH_3 concentrations increased in *O. niloticus* reared at high density in tanks (Dawood et al. 2020). High stocking rates in intensive farming systems can adversely affect water quality. Several reasons contribute to this finding, such as the accumulation of metabolic waste products resulting from the respiration and excretion of densely stocked fish (Boyd 1990). The decomposition of these waste products may result in relatively high levels of carbon dioxide, TAN, and NH_3. As a result, this will increase NH_3 emissions and decrease DO levels in the rearing water (Boyd and Tucker 2012). Moreover, the increased stocking densities will lower the water circulation in culture units and reduce the DO level (Yi and Kwei Lin 2001). In this case, wasteful feeding must always be avoided because the uneaten feed has a much higher impact on water quality than eaten feed and will speed up the development of adverse culture conditions (Boyd 1982).

The growth parameters of *P. hypophthalmus* were decreased alongside the increase in the stocking densities. In the same fish species, it was found that the growth parameters were significantly reduced in *P. hypophthalmus* fingerlings reared under HSD rates (Malik et al. 2014). Likewise, it was reported that WG and SGR significantly decreased in *P. hypophthalmus* raised in high density in floodplain cage culture systems (Chowdhury et al. 2020). It was demonstrated that *P. hypophthalmus* fingerling growth dropped in biofloc rearing tanks at a stocking density of 270 fish/m^3 or above in biofloc culture systems (Nageswari et al. 2022). It was also noted that HSD also depressed the growth of *O. mykiss* (Liu et al. 2016), *I. punctatus* fingerlings (Refaey et al. 2018), *O. niloticus* (Dawood et al. 2020), and *C. gibelio* (Onxayvieng et al. 2021). Interestingly, it was found that FI, PER, PPV, and EU values in the low-density group (SD60) were higher than in the other groups; meanwhile, the FCR in the SD6 group was markedly lower than in the SD120 and SD150 groups. This was similar to Chowdhury et al. (2020), who showed that low-density cage culture of *P. hypophthalmus* (19 fish/m^3) resulted in better feed utilization efficiency. Moreover, better FCR values were recorded in *P. hypophthalmus* fingerlings reared in low density (Malik et al. 2014).

Herein, we found that the optimum stocking rate was 60 fish/m^3 compared with HSD groups (90, 120, and 150 fish/m^3, respectively). These results were built upon the higher growth rates recorded after studying the relationships between the different stocking densities and growth indices (FW, FI, WG%, and SGR). These findings differed from other studies conducted on *Pangasiidae* fish species. For instance, it was reported that better growth and yield were obtained when *Pangasius sutchi* was reared in a density of 150 fish per 1 m^3 in

cages (Islam et al. 2006; Rahman et al. 2006). Moreover, it has been described that the SGR was not significantly differed between groups when the stocking densities of the hapa-reared *Pangasius bocourti* increased to 150–200 fish/m^3 (Jiwyam et al. 2010; Jiwyam 2011). Malik et al. (2014) showed that rearing *P. hypophthalmus* fries in tanks or ponds with a stocking density of 100 fries/m^3 were suitable for better growth performance. Chowdhury et al. (2020) illustrated that rearing *P. hypophthalmus* in a density of 22 fish per 1 m^3 in floodplain cages is optimum for better growth and productivity. A recently published paper showed that the optimum density rate for *P. hypophthalmus* fingerlings reared in a BFT-based unit can be increased to reach 210–240 fish/m^3 (Nageswari et al. 2022). The differences between these studies and our findings may be attributed to several reasons, such as the culture system (hapas, ponds, tanks, cages, or BFT system), size and age of the cultured fish species (fries, fingerlings, or larger fish), the experimental design (rearing period and diet composition), or different localities with different environmental conditions.

On the other hand, the previously published literature showed that low stocking density (LSD) in the culture facilities would give the farmed fish more space for proper movement, decrease fish competition for food, and subsequently induce better feed efficiency and utilization (Slembrouck et al. 2009; Refaey et al. 2018). Moreover, the fish reared in LSD will be less stressed than those raised in HSD (Zahedi et al. 2019). These factors will help to increase fish growth rates (Rahman et al. 2006). Notably, fish growth depression in HSD may be associated with several causes, such as deteriorated water quality, as in our study, which will lead to depressed growth rates (Boyd and Tucker 2012). Another possible reason is the decreased thyroid hormone levels in fish blood (Li et al. 2012; Refaey et al. 2018). Moreover, rearing fish in HSD will increase the energy demands and requirements for various physiological responses of fish to survive during stressful conditions of high fish numbers per space unit and subsequently will lead to a decline in the available energy required for growth (Wendelaar Bonga 1997; Qi et al. 2016). Besides, HSD rates (high number of fish per space unit) will minimize the space necessary for fish movement, increase the competitiveness of fish for food, and consequently will lead to a decreased feed consumption, and thus reduce the FW, WG, ADG, and WG% (Duan et al. 2011). Regarding the fish survival in the present study, there were density-dependent mortalities among the experimental groups. It was found that *P. hypophthalmus* fingerlings reared at HSD, especially in the SD150, had lower survival percentages than others. This finding may be associated with the higher competition for food and required space among fish (Malik et al. 2014).

The organ-somatic indices such as VSI, KSI, HSI, and SSI of *P. hypophthalmus* fingerlings had the lowest values in the fish group with the HSD rate (SD150). In a similar trend, it was found that KSI, HSI, and SSI were significantly increased in *Acipenser schrenckii* juveniles reared at LSD rate (Ni et al. 2014). Moreover, higher HSI (%) was also found in hybrid grouper juveniles reared at LSD rate (Shao et al. 2019). Our findings may be attributed to improved growth performance and welfare in the fish group with a low stocking rate (SD60), which will subsequently enhance the organ somatic indices of fish.

The highest moisture and ash and the lowest CP, EE, and carcass energy contents were recorded in the whole fish body in the SD150 group with the HSD rate. These findings may be due to HSD causing chronic stress in the reared fish, which may impact the fish's body composition. Our results were in concordance with those described by Onxayvieng et al. (2021), who found that the moisture and ash (%) were the highest and the EE (%) was lowest in the musculature of gibel carp reared at the HSD rate. Differently, it was shown that the musculature of channel catfish reared at the HSD rate had the lowest EE and the highest CP in comparison with those raised under LSD rates (Refaey, et al. 2018). Moreover, the study on *Clarias gariepinus* and *Heterobranchus longifilis* showed that the CL% of

the carcass increased with the stocking density, while carcass CP and moisture (%) were decreased (Toko et al. 2007). The inconsistencies in results might be related to fish species, experimental design, rearing period, or others.

The TP, ALB, and lysozyme activity were significantly decreased; meanwhile, glucose, cortisol, BUN, ALT, AST, and ALP levels of *P. hypophthalmus* fingerlings were elevated alongside the increase in the stocking rates. Nageswari et al. (2022) declared that higher TP, ALB, and GLO were found in *P. hypophthalmus* fingerlings reared in LSD compared with those raised in HSD in BFT-based units. In other fish species, it was noted that serum glucose, triglycerides, and cholesterol, as well as ALT and AST activities, were significantly increased in *I. punctatus* fingerlings as stocking density increased (Refaey et al. 2018). Blood glucose and cortisol values were also increased, and lysozyme activities were significantly decreased in Nile tilapia reared under HSD rates (Dawood et al. 2019, 2020). Similarly, serum cortisol levels and ALT, AST, and ALP activities were increased considerably as the stocking densities increased in *Megalobrama amblycephala* juveniles (Wang et al. 2019a). Also, the highest serum ALT, ALP, AST, and lysozyme activities were recorded in Nile tilapia fingerlings reared in HSD in BFT-based systems (Liu et al. 2018). Likewise, plasma ALT, AST, glucose, and cortisol levels were significantly elevated in hybrid grouper juveniles reared under HSD densities in the RAS system (Shao et al. 2019). A recent study showed that blood glucose, cortisol, ALT, AST, cholesterol, and creatinine were elevated in gibel carp reared under HSD rates (Onxayvieng et al. 2021). On the contrary, no changes were recorded in serum ALT, AST, ALP, cortisol, and lysozyme in GIFT tilapia raised under different densities in the pond raceway RAS system (Wang et al. 2019b). This study has differed from other density studies due to various fish species, rearing systems, number of fish per space unit, experimental design, and others.

Generally, it was well-known that blood proteins such as ALB, GLO, and TP are effective bioindicators of fish humoral immunity (Patriche et al. 2009; Andreeva 2010). Lysozyme is important in non-specific fish immunity (Alexander and Ingram 1992; Saurabh and Sahoo 2008). Blood glucose is usually increased for energy production to minimize the impacts of stress on the exposed fish (Wendelaar Bonga 1997). Likewise, high cortisol levels in the serum evoke fish exposure to various stressors (Barton and Iwama 1991; Barton 2002). Moreover, the elevation of both blood glucose and cortisol values may be associated with the activation of glycolysis in the liver to produce glucose as an energy source for fish reared under stress (Barton and Iwama 1991; Martínez-Porchas et al. 2009). Thus, blood glucose and cortisol are stress markers of fish (Eissa et al. 2022; Khalil et al. 2022a). Interestingly, ALT, ALP, and AST are biomarkers of fish liver functions (Abdel-Latif et al. 2020b), and their elevation in fish blood circulation suggests the occurrence of hepato-renal injury (Bruslé and Anadon 1996). Besides, the elevation of BUN levels indicates renal and gill dysfunction (Nelson et al. 1999). Taken together from the abovementioned findings in our experiment, we suggest that *P. hypophthalmus* fingerlings reared at HSD were stressed and exhibited hepato-renal and gill dysfunctions and depressed immunity.

The endogenous enzymatic antioxidant mechanisms of the fish body can defend the host against oxidative stress and alleviate the adverse impacts of free radicals (Chowdhury and Saikia 2020). MDA is a lipid peroxidation biomarker, and T-AOC capacity is a biomarker of the antioxidant capacity of fish tissues. The HSD of *P. hypophthalmus* fingerlings in the SD150 group significantly decreased CAT, SOD, and T-AOC levels and significantly increased MDA and GPx levels compared with other experimental groups. These findings suggest that fish reared under HSD in the SD150 group had a depressed antioxidant capacity and increased susceptibility of fish to oxidative stress. These findings may be occurred because of the continuous chronic stress of overcrowding of fish in the culture units, which lead to the overproduction of

radicals and hinder the endogenous defensive mechanisms of fish and finally lead to oxidative damage (Costas et al. 2013). Our results are in concordance with Dawood et al. (2019), who declared that the antioxidant biomarkers were depressed in Nile tilapia reared under intensive densities. Also, HSD caused significant depression of hepatic CAT and reduced glutathione (GSH) levels in hybrid grouper juveniles (Shao et al. 2019). Hepatic SOD and GPx were decreased, and MDA concentrations were increased with the stocking rates of *M. amblycephala* juveniles (Wang et al. 2019a). Hepatic SOD and CAT were also decreased, and MDA contents were increased in largemouth bass (*Micropterus salmoides*) reared under the HSD rate (Ni et al. 2021). Onxayvieng et al. (2021) highlighted that gibel carps raised under the LSD rate had the highest SOD, GPx, and CAT enzyme activities and GSH and T-AOC levels compared with those reared under the HSD rate. Although the studies mentioned above found hepatic SOD, CAT, GPx, and MDA levels showed no significant differences in GIFT tilapia raised under different densities in the pond raceway system (Wang et al. 2019a).

Digestive enzymes reflect fish's ability to digest and assimilate the ingested food. The intestinal lipase, amylase, trypsin, and protease enzymes were decreased with the stocking density rates. Similarly, it was found that the intestinal amylase, lipase, and protease enzymes were significantly decreased in *M. amblycephala* juveniles reared under the HSD rate (Wang et al. 2019a). Moreover, trypsin, amylase, and lipase enzyme activities were significantly depressed in Nile tilapia fingerlings raised in HSD in a BFT-based system (Liu et al. 2018). Similarly, the amylase, lipase, and protease enzymes were increased in *O. niloticus* reared at an LSD rate (Dawood et al. 2019). Also, intestinal amylase, lipase, and trypsin enzyme were decreased in *M. salmoides* reared at an HSD rate in a pond raceway system (Ni et al. 2021). However, no variations were noticed in the lipase, amylase, and protease enzymes in the stomach and intestine of GIFT tilapia raised under different densities in the RAS system (Wang et al. 2019b). Different fish species, culture systems, number of fish per space unit, and experimental design are the factors that may affect the impacts of stocking densities on digestive enzymes. The possible reasons for the changes that occurred in our study because of HSD may be attributed to chronic stress-induced because of overcrowding of fish in the culture units, which may disrupt the fish endocrine system, increase the cortisol levels, and depress the intestinal digestive enzyme activity (Ni et al. 2021). Notably, the results of intestinal digestive enzymes in *P. hypophthalmus* fingerlings reared at different stocking densities are closely related to the effects of growth parameters. This means that at LSD rates of fish in the group SD60, there were higher fish growth rates and higher digestive enzyme activities.

Conclusions

From the findings, we noticed that rearing fish in adequate ambiance would provide a suitable environment for farming fish in higher stocking densities. Moreover, improving the ambiance conditions for the striped catfish is crucial to maintaining or improving their productive performance. Furthermore, we can conclude that stocking fish in higher densities negatively affected the water quality parameters, which, in turn, influenced the surrounding environment of the fish and thus led to poor performances and deteriorated health indicators of *P. hypophthalmus* fingerlings. To put it briefly, a stocking rate of 60 fish/m^3 can be considered an optimum rate for tank-reared *P. hypophthalmus* fingerlings for better growth, biometric indices, body composition, serum immunity, stress biomarkers, hepatic antioxidants, and digestive enzymes without deterioration of the quality of the rearing water.

Author contributions Mohamed A.A. Zaki: Conceptualization, Writing – review, Formal analysis.Hala Saber Khalil: Investigation, Methodology, Data curation, Writing - review & editing.Belal W. Allam: Methodology, Validation, Investigation.Riad H. Khalil: Methodology, Validation, Investigation.Mohammed F. El Basuini: Methodology, Validation, Investigation.Abd El-Aziz M. Nour: Data curation, Investigation. Eman M.H. Labib: Data curation, Formal analysis, Software.Islam S.E. Elkholy: Methodology, Validation, Investigation.Marc Verdegem: Data curation, Writing - review & editing.Hany M.R. Abdel-Latif: Data curation, Writing – original draft, Writing - review & editing.

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Data Availability The data are available upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The works conducted in the present study have been certified by the Local Experimental Animal Care Committee, Faculty of Veterinary Medicine, Alexandria University, and approved by the Institutional Animal Care and Use Committee at Alexandria University (ALEXU-IACUC) with ethical Approval Code (AU-013/2022/11/-3R/4P/158).

Conflict of interest The authors declare no competing interests.

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