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HIGH STOCKING DENSITY AFFECTED THE GROWTH PERFORMANCE, DIGESTIVE ENZYME ACTIVITY, INTESTINAL HISTOLOGY, AND IMMUNE RESPONSE OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FRY GROWN IN BRACKISH WATER

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Abstract

It is crucial to ensure the suitable stocking density for Nile tilapia fry since Nile tilapia is among the most consumed fish species globally. In this study, fry were distributed at three stocking densities, 1000 fry/m^3 (low density, LD), 2000 fry/m^3 (middle density, MD), and 4000 fry/m^3 (high density, HD). Then each stocking density was subdivided into two groups where three aquaria were supplied with fresh dechlorinated water (FW, 0.35 g saline/L), and the other three aquaria were fortified with underground brackish water (BW, ≈ 8 g saline/L). Subsequently, fry were kept under these experimental conditions and offered 40% crude protein thrice daily at 4–5% of the body mass for 15 days, then switched to 30% thrice daily at 3–4% of the body mass until the end of the trial (60 days). Except for the dissolved oxygen, salinity, and total ammonia nitrogen, the measured water characteristics (temperature and pH) were not markedly (P>0.05) affected by the stocking density or water salinity. Stocking density was a significant factor in the case of final body weight, weight gain, specific growth rate, and survival rate (P<0.05). The water salinity and stocking density markedly affected the digestive enzyme activity (protease, lipase, and amylase) (P<0.05). The stocking density, water salinity, and their interaction were significant factors in the lysozyme activity. The stocking density significantly affected the superoxide dismutase (SOD), catalase activity (CAT), and glutathione peroxidase (GPx), and fish fry in LD showed higher SOD, CAT, and GPx than fish in MD or HD in FW or BW (P<0.05). Malondialdehyde (MDA) was affected by the stocking density, and fish fry in LD showed lower MDA than in MD or HD under FW or BW (P<0.05). Increased density to HD in the FW or BW induced slight degeneration of the intestinal mucosal lining. In conclusion, Nile tilapia fry can grow in brackish water (≈ 8 g saline/L) with 1000 to 2000 fry/m^3 without affecting growth performance, feed utilization, digestive enzymes, intestinal histological features, and immune and antioxidative responses.

Key words: digestion, health status, intensive farming, tilapia, water scarcity

Nile tilapia (*Oreochromis niloticus*) is the third largest farmed fish species globally, with a total production of over 4.5 million tons annually (FAO, 2022). Nile tilapia presents a sustainable fish farming solution associated with a high tolerance to biotic and abiotic stressors (Xiong et al., 2023). Growing Nile tilapia in high stocking density has also been approved, leading to high productivity that could cover humanity's consumption (Wang et al., 2023). Therefore, Nile tilapia has been successfully cultured in more than 120 countries (Xiong et al., 2023). Nile tilapia is also an euryhaline fish species with a high ability to tolerate a wide range of water salinities (Larumbe-Morán et al., 2010).

Nile tilapia can tolerate stocking-density farming systems, which may lead to stress features and impaired physiological responses (Huang and Chiu, 1997). Growing Nile tilapia under high stocking density was cautiously implemented, especially in optimizing the water quality indices (Kabir et al., 2019). Therefore, the proper farming conditions for Nile tilapia farming are highly recommended to overcome the environmental stressors that coincide with high stocking density (El-Sayed and Fitzsimmons, 2023; Furuya et al., 2023). Proper water salinity, dissolved oxygen, ammonia levels, pH, and temperature are the main characteristics of quality assurance for feasible Nile tilapia farming (MacKinnon et al., 2023). Growing Nile tilapia in brackish water has been confirmed where fish can perform optimally up to 10–15 g saline/L (Durigon et al., 2020; Ninh et al., 2014). It is well documented that the adaptation of euryhaline species to improper water salinity causes impacts on the osmoregulatory and metabolic function and, thereby, the general physiological and health status of fish (Fridman et al., 2012; Yan et al., 2013). Consequently, the energy cost required for osmoregulation decreases the growth rate and productivity (Febry and Lutz, 1987; Tseng and Hwang, 2008). Several trials have been conducted to investigate the culture of Nile tilapia in brackish water, and the response of fish was dependent on the life stage, sizes, stocking density, and environmental conditions

(Garg et al., 2023; Mashaii et al., 2016; Shao et al., 2023; Yousefi et al., 2022). Hence stocking density and water salinity were crucial factors that require close attention since Nile tilapia is a suitable fish for arid areas suffering from a lack of freshwater.

Egypt is a leading country in Nile tilapia production, with a total production of over two million tons in 2022 (GAFRD, 2021; Rossignoli et al., 2023), making Egypt the third-largest tilapia producer in the whole world (FAO, 2022). However, the high competition in the agriculture sector and human consumption affects the availability of freshwater resources in the aquaculture sector (Ibrahim et al., 2023; Sewilam et al., 2023). Therefore, underground water was practiced in many hatcheries and farms involved in Nile tilapia production (Mehrim and Refaey, 2023). Keeping Nile tilapia fry healthy requires wide ponds, suitable water resources, and optimum stocking density to ensure suitable conditions for fish nursing. However, commercial, and private hatcheries produce large numbers of fry (>3 g/fry), which leads to stocking fish under high stocking densities and may result in high mortality rates. Besides, brackish water may influence the metabolic and physiological responses of Nile tilapia fry reared in high stocking densities.

The responses of Nile tilapia juveniles under different stocking densities have been investigated in many studies. In this regard, Abdel-Tawwab et al. (2014), Zafar et al. (2022) and Liu et al. (2018) reported that high stocking density could affect the growth performance, health status, and digestion capacity of Nile tilapia juveniles when grown in freshwater or brackish water. Further, Luz et al. (2012) reported that Nile tilapia larviculture (30 larvae/L) was grown successfully with high stocking density at 2 g saline/L. However, stocking Nile tilapia fry under high stocking density with brackish water has not been investigated yet. Hence the current study aimed to test the survival rate, growth performance, intestinal health, and immune and antioxidative responses of Nile tilapia grown in brackish water (≈ 8 g/kg) under different stocking densities.

Material and methods

Experimental design and procedure

Mono-sexed fry of Nile tilapia (*Oreochromis niloticus*) with 2.85±0.14 g/fry were obtained from the hatching unit of the Egyptian Aquaculture Center for Training and Applied Research (EACTAR), Kafrelsheikh, Baltim highway, Kafrelsheikh governorate, Egypt. The fry were moved gently to the experimental wet laboratory at EACTAR and stocked in eighteen glass aquaria (70 L/aquarium). Fry were adapted to the experimental conditions for one week, where each aquarium was supplied with fresh dechlorinated water and continuous aeration. Fish received commercial feeds (Aller-Aqua Co., Egypt) with 40% crude protein and 6% crude lipids thrice daily at 6–8% of the body mass. The water was exchanged daily at 60% of the total capacity for aquaria with newly fresh chlorine-free water. Afterward, fry were redistributed among the eighteen aquaria at three stocking densities, where six aquaria were stocked with 55 fry/aquarium. The remaining twelve aquaria were divided into two groups (six in each), where six aquaria stocked with 110 fry/aquaria and six aquaria stocked with 220 fry/aquarium. Although the aquarium capacity is 70 L/aquarium, the actual water content was 55 L/aquarium; therefore, the stocking densities for the three groups were based on 1000 fry/m^3 (low density, LD), 2000 fry/m^3 (middle density, MD), and 4000 fry/m^3 (high density, HD). Then each group of the three stocking densities was subdivided into two groups where three aquaria were supplied with fresh dechlorinated water (FW, 0.35 g saline/kg), and the other three aquaria were fortified with underground brackish water (BW, ≈ 8 g saline/kg). The study design is factorial (2×3) with two salinity levels (FW and BW) and three stocking densities (LD, MD, and HD). Fry in the BW groups gradually adapted to the brackish water by mixing the aquaria water with brackish water (≈ 8 g saline/kg) daily during water exchange. Adapting to the BW conditions lasted for three days to avoid stressful conditions for fish. The BW was sourced from underground water with about 8 g saline/kg salinity. Subsequently, fry were kept under these experimental conditions and offered 40% crude protein thrice daily at 4–5% of the body mass for 15 days, then switched to 30% thrice daily at 3–4% of the body mass until the end of the trial (60 days). During the trial, the amount of feed was recorded for each aquarium, and water quality measurements were detected, especially for the water salinity, which was checked daily. Besides, the survival rate was monitored, and dead fish were removed accordingly.

Water quality measurement

Water quality indices were checked and recorded by Orion Star™ A329 Portable Multiparameter Meter (Thermo Scientific™, Waltham, Massachusetts, U.S.) for salinity, temperature, dissolved oxygen, and pH. Total ammonia nitrogen was estimated using a test kit (Model NI, Cat. No. 22669-00, Hach Co.).

Final sampling

All fish were fasted for 24 hours at the end of the trial, then weighed and counted. The following equations were used to calculate the weight gain (WG), specific growth rate (SGR), feed intake (FI), daily feed intake (DFI), and feed conversion ratio (FCR):

$$WG\ (g) = [final\ body\ weight,\ FBW\ (g) - initial\ weight,\ IBW\ (g)] / IBW\ (g)$$

$$SGR\ (\%/day) = (\ln FBW - \ln IBW) / duration\ (60\ days) \times 100$$

$$DFI = FI\ (g) / days$$

$$FCR = FI\ (g) / WG\ (g)$$

$$Survival\ (\%) = 100 \times final\ fish\ number / initial\ fish\ number$$

Subsequently, five fish per aquarium were collected, anesthetized (100 mg/L tricaine methanesulfonate), and bled from the caudal vein by 2.5 mL syringes. The collected blood was kept in non-heparinized tubes for serum collection. Blood was kept for 3 hours to clot, and centrifuged at 3000 rpm for 15 min at 4ºC to separate the serum. Intestines were gently dissected from 3 fish per aquarium and kept in formalin (10%) for the histological examination. Additionally, fish were dissected, and the livers were collected and weighed to calculate the hepatosomatic index (HSI). Three fish per tank were collected and weighed, and their body length was recorded to calculate the condition factor (CF).

$$CF = \text{body weight (g)} / (\text{fish length})^3\ (\text{cm})^3 \times 100$$

$$HSI\ (\%) = \text{liver weight (g)} / \text{body weight (g)} \times 100$$

Digestive enzyme activity

Digestive enzyme activity was determined in the serum samples. Lipase was determined colorimetrically at the wavelength 580 nm (Spectrum, Egyptian Company for Biotechnology, Egypt) by following Moss and Henderson (1996). Amylase activity was determined colorimetrically at the wavelength 660 nm using commercial kits (Biodiagnostic Co., Egypt) by following Caraway (1959). Protease activity was determined colorimetrically at the wavelength 450 nm following the manufacturer's instructions (Thermo Scientific, Egypt).

Blood analysis

Lysozyme activity was assayed by the ELISA microwell technique using fish lysozyme ELISA kit (Sunlong Biotech Co., China) at the wavelength 450 nm. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured using diagnostic reagent kits following the manufacturer's (Biodiagnostic Co., Egypt) instructions. The concentration of malondialdehyde (MDA) was detected by adhering to the advice of Uchiyama and Mihara (1978) and expressed as nmol MDA/mL.

Histological analysis

The middle intestine samples were immediately fixed in 10% formalin solution then dehydrated through ascending grades of an alcohol solution series, cleared in xylene, embedded in paraffin, cut into several 5-µm-thick sections using a rotary microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany), and mounted on coated slides (Bancroft et al., 1996). The prepared paraffin sections were rehydrated and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope (Leica DM500; Leica Microsystems, Japan).

Statistical analysis

Shapiro-Wilk and Levene's tests confirmed normal distribution and homogeneity of variance. The obtained data were subjected to one-way ANOVA. Differences between means were tested at $P<0.05$ level using the Duncan test as a post-hoc test. Two-way ANOVA (P-value) was also applied to check the effects of water salinity, stocking density, and their interaction. All the statistical analyses were done via SPSS version 22 (SPSS Inc., IL, USA). *t*-test was applied to check the significant differences for each of the three stocking densities with freshwater and brackish water.

Table 1. Water quality indices during the trial

Group	Dissolved oxygen (mg/L)	Temperature (°C)	pH	Total ammonia nitrogen (mg/L)	Salinity (g/L)
FW/LD	5.41±0.11 a	27.65±0.54	7.45±0.21	0.21±0.03 c	0.28±0.05 b
FW/MD	5.38±0.21 a	27.64±0.47	7.44±0.14	0.23±0.01 c	0.44±0.06 b
FW/HD	4.12±0.32 b	27.62±0.38	7.46±0.32	0.51±0.02 b	0.45±0.24 b
BW/LD	5.28±0.28 a	27.68±0.61	7.36±0.42	0.21±0.01 c	7.91±0.34 a*
BW/MD	5.09±0.15 a	27.57±0.49	7.41±0.29	0.44±0.02 b	7.94±0.42 a*
BW/HD	4.11±0.31 b	27.68±0.54	7.48±0.36	0.62±0.02 a*	7.99±0.61 a*
Two-way ANOVA (P-value)					
water salinity (WS)	0.506	0.422	0.429	0.922	0.0001
stocking density (SD)	0.011	0.472	0.427	0.004	0.521
WS × SD	0.081	0.631	0.361	0.367	0.364

Means ± SE (n = 3) with different letters in the same column show significant differences among the whole groups ($P<0.05$). The asterisk symbol (*) refers to significant differences ($P<0.05$) between fish reared in freshwater and brackish water using a *t*-test. FW: freshwater; BW: brackish water; SD: stocking density; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3).

Table 2. Growth performance of Nile tilapia fry reared under different stocking densities in freshwater or brackish water for 60 days

Group	IBW	FBW	WG (%)	SGR	CF	HSI	Survival (%)
FW/LD	2.68±0.04	9.90±0.65 a	269.76±18.91 ab	2.17±0.09 a	1.71±0.06	3.22±0.15	91.92±2.76 a
FW/MD	2.64±0.03	7.10±0.19 b	169.30±6.08 b	1.65±0.04 b	1.79±0.06	3.57±0.49	84.61±0.64 b
FW/HD	2.38±0.01	5.77±0.01 c	141.47±1.37 c	1.47±0.01 c	1.75±0.06	2.85±0.04	80.44±1.20 c
BW/LD	2.66±0.04	10.43±0.42 a	292.03±20.04 a*	2.27±0.09 a	1.74±0.08	3.26±0.46	92.09±1.18 a
BW/MD	2.68±0.04	7.14±0.08 b	166.40±2.64 b	1.63±0.02 b	1.86±0.06	3.09±0.63	87.62±0.96 b
BW/HD	2.42±0.04	6.26±0.03 c	159.19±3.41 c*	1.59±0.02 bc	1.81±0.14	2.93±0.55	83.41±0.28 b
Two-way ANOVA (P-value)							
water salinity (WS)	0.503	0.21	0.294	0.201	0.434	0.743	0.099
stocking density (SD)	0.624	0.0001	0.0001	0.0001	0.533	0.589	0.0001
WS × SD	0.744	0.708	0.635	0.505	0.971	0.781	0.529

Means ± SE ($n = 3$) with different letters in the same column show significant differences among the whole groups ($P<0.05$). The asterisk symbol (*) refers to significant differences ($P<0.05$) between fish reared in freshwater and brackish water using a *t*-test. FW: freshwater; BW: brackish water; SD: stocking density; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3); IBW: initial body weight; FBW: final body weight; WG: weight gain; SGR: specific growth rate; CF: condition factor; HSI: hepatosomatic index.

Table 3. Feed utilization of Nile tilapia fry reared under different stocking densities in freshwater or brackish water for 60 days

Group	FI (g/fish/60 days)	DFI (g/fish/day)	FCR
FW/LD	11.26±0.82 a	0.19±0.01 a	1.56±0.03 b
FW/MD	10.89±0.31 a	0.18±0.01 a	2.44±0.06 a
FW/HD	8.79±0.21 b	0.15±0.02 b	2.60±0.05 a
BW/LD	10.92±0.25 a	0.18±0.02 a	1.41±0.05 b
BW/MD	10.67±0.27 a	0.18±0.01 a	2.39±0.05 a
BW/HD	9.86±0.27 b	0.16±0.03 b	2.57±0.08 a
Two-way ANOVA (P-value)			
water salinity (WS)	0.184	0.114	0.149
stocking density (SD)	0.0001	0.0001	0.0001
WS × SD	0.071	0.064	0.645

Means ± SE ($n = 3$) with different letters in the same column show significant differences among the whole groups ($P<0.05$). FW: freshwater; BW: brackish water; SD: stocking density; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3); FI: feed intake; DFI: daily feed intake; FCR: feed conversion ratio.

Results

Water quality

The water quality indices in the rearing aquaria of Nile tilapia fry grown under low density (LD), middle density (MD), and high density (HD) in freshwater (FW) or brackish water (BW) are shown in Table 1. Except for the dissolved oxygen (DO), salinity, and total ammonia nitrogen (TAN), the measured water characteristics (temperature and pH) were not markedly (P>0.05) affected by the stocking density, water salinity, and their interaction. However, water salinity was significantly impacted by the FW and BW factors, where water in FW (0.28–0.45 g saline/L) was significantly lower in salinity than water in BW groups (7.91–7.99 g saline/L) (P<0.05). The level of DO was significantly affected by the stocking density, where fish fry in HD had lower DO levels than LD groups and grown in FW or BW (P<0.05). In addition, TAN was significantly affected by the stocking density, where fish fry in HD had higher total ammonia levels than LD groups and grown in FW or BW (P<0.05). Besides, the water in the case of BW and HD (0.41 mg/L) has higher TAN than in the water of FW with HD (0.41 mg/L) (P<0.05). The remaining water indices showed the following values for the temperature (27.57–27.65°C) and pH (7.36–7.48), without significant differences.

Growth performance, survival rate, and somatic indices

Growth performance, survival rate, and somatic indices of Nile tilapia fry reared under different stocking densities in FW or BW for 60 days are shown in Table 2. Stocking density was a significant factor in the case of final body weight (FBW), weight gain (WG), specific growth rate (SGR), and survival rate (P<0.05), while water salinity was not a significant factor (P>0.05). In this regard, fish fry in groups reared in FW or BW in LD showed higher FBW, WG, SGR, and survival rates than in MD or HD (P<0.05). In addition, fish fry in MD showed higher FBW, WG, SGR, and survival rates than fish fry in HD (P<0.05). Surprisingly, the WG of fish fry in LD or HD under BW is higher than LD or HD in FW (P<0.05). The CF and HSI were not significantly affected by the water salinity, stocking density, and their interaction (P>0.05).

Feed utilization

The feed intake (FI), daily feed intake (DFI), and feed conversion ratio (FCR) of Nile tilapia fry reared under different stocking densities in FW or BW for 60 days were significantly affected by the stocking density (P<0.05), but the water salinity was not a significant factor (P>0.05) (Table 3). Fish fry in FW or BW in LD or MD showed higher FI and DFI than in HD (P<0.05). While fish fry in HD showed higher FCR than in LD or MD (P<0.05).

Digestive enzyme activity

The digestive enzyme activities of Nile tilapia fry reared under different stocking densities in FW or BW for 60 days are shown in Table 4. The water salinity and the interaction between water salinity and stocking density markedly affected the protease activity (P<0.05). Fish fry grown in BW under LD, MD, and HD showed higher protease activity than fish fry in FW under MD and HD (P<0.05). The lipase activity was affected markedly by the interaction between water salinity and stocking density, and fish fry in LD or MD showed higher activity than fish fry in HD either in FW or BW (P<0.05). The stocking density affected amylase activity significantly, and fish fry in LD or MD showed higher activity than fish in HD, either in FW or BW (P<0.05).

Table 4. Digestive enzyme activity of Nile tilapia fry reared under different stocking densities in freshwater or brackish water for 60 days

Group	Protease activity (unit/mg)	Lipase activity (unit/mg)	Amylase activity (unit/mg)
FW/LD	22.305±1.94 b	36.0767±3.05 a	26.02±3.16 a
FW/MD	14.37±2.71 d	34.79±1.26 a	25.3433±2.54 a*
FW/HD	13±2.20 d	26.855±0.28 b	18.84±1.83 b*
BW/LD	29.68±6.18 a*	35.9±2.64 a	26.5633±1.74 a
BW/MD	23.7567±3.28 b*	33.7467±3.12 a	20.8167±0.47 b
BW/HD	19.4467±0.27 c*	27.8667±2.75 b	12.7867±0.89 c
Two-way ANOVA (P-value)			
water salinity (WS)	0.014	0.973	0.062
stocking density (SD)	0.77	0.148	0.001
WS × SD	0.035	0.027	0.262

Means ± SE (n = 3) with different letters in the same column show significant differences among the whole groups (P<0.05). The asterisk symbol (*) refers to significant differences (P<0.05) between fish reared in freshwater and brackish water using a t-test. FW: freshwater; BW: brackish water; SD: stocking density; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3).

Table 5. Blood immunity and antioxidative responses of Nile tilapia fry reared under different stocking densities in freshwater or brackish water for 60 days

Groups	Lysozyme activity (unit/ml)	Superoxide dismutase (IU/L)	Catalase (IU/L)	Glutathione peroxidase (IU/L)	Malondialdehyde (nmol/ml)
FW/LD	4.41±0.36 a	29.06±1.59 a*	30.08±0.52 a	13.09±0.84 a*	18.46±1.61 b
FW/MD	2.86±0.12 b	23.76±0.72 b*	28.84±1.38 a	11.47±0.73 a*	31.29±0.41 a
FW/HD	2.02±0.09 c	21.19±0.67 b	19.73±0.50 b	8.76±0.91 b	30.62±0.57 a
BW/LD	3.06±0.10 ab	25.83±1.43 ab	26.20±1.41 ab	12.15±0.64 a	19.31±0.39 b
BW/MD	2.59±0.25 b	21.81±1.31 b	27.09±1.07 a	9.80±0.87 b	29.30±1.39 a
BW/HD	1.98±0.08 c	19.34±01.26 b	17.84±1.65 b	8.14±0.12 b	29.13±0.85 a
Two-way ANOVA (P-value)					
water salinity (WS)	0.001	0.437	0.806	0.916	0.413
stocking density (SD)	0.0001	0.025	0.003	0.041	0.007
WS × SD	0.005	0.174	0.441	0.747	0.614

Means ± SE (n = 3) with different letters in the same column show significant differences among the whole groups (P<0.05). The asterisk symbol (*) refers to significant differences (P<0.05) between fish reared in freshwater and brackish water using a t-test. FW: freshwater; BW: brackish water; SD: stocking density; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3).

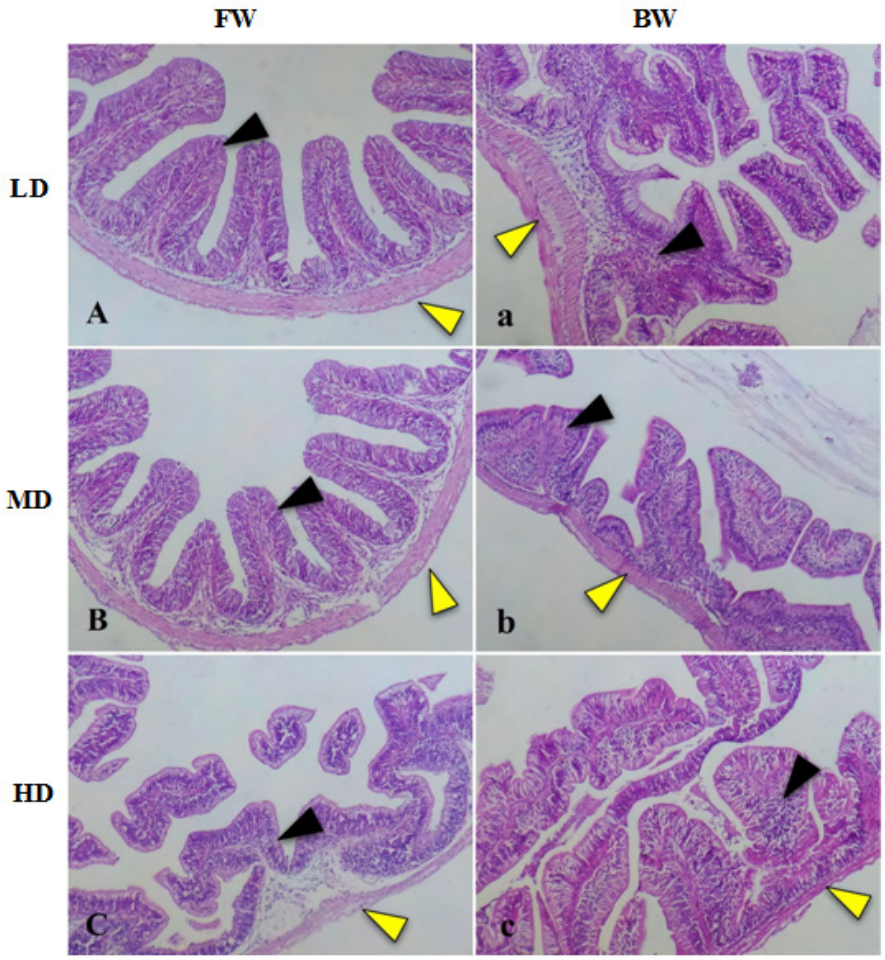


Figure 1. Histomicrograph of the middle segment of the intestine of Nile tilapia reared in fresh (A, B, C) and brackish water (a, b, c) at different densities. A and B revealed the normal histological structure of both intestinal mucosa (black arrow) and intestinal wall (yellow arrowhead). C and c showed degeneration of intestinal villi. The high stocking density groups revealed degeneration, inflammatory cell infiltration in the lamina propria (C, c), and hyperplasia of the intestinal wall. Stain H&E. Bar = 100 µm. FW: freshwater; BW: brackish water; LD: low density (1000 fry/m^3); MD: middle density (2000 fry/m^3); HD: high density (4000 fry/m^3)

Blood immunity and antioxidative responses

Blood immunity and antioxidative responses of Nile tilapia fry reared under different stocking densities in FW or BW for 60 days are shown in Table 5. The stocking density, water salinity, and their interaction were significant factors in the lysozyme activity. Fish fry in groups reared in FW or BW in LD and MD showed higher lysozyme activity than in HD ($P<0.05$). In addition, fish fry in MD showed higher lysozyme activity than in LHD ($P<0.05$). The stocking density significantly affected the superoxide dismutase (SOD), and fish fry in LD showed higher SOD than fish in MD or HD in FW or BW ($P<0.05$). The stocking density significantly affected the catalase activity (CAT), and fish fry in LD or MD showed higher CAT than fish in HD in FW or BW. The stocking density also affected the glutathione peroxidase (GPx), and fish fry in LD or MD showed higher GPx than in HD ($P<0.05$). Malondialdehyde (MDA) was affected by the stocking density, and fish fry in LD showed lower MDA than in MD or HD under FW or BW ($P<0.05$).

Histopathological findings

The histological study of Nile tilapia intestine illuminated the normal structure of both intestinal mucosa and intestinal wall throughout the whole length of the middle intestine in the FW or BW at LD and MD (Figure 1). Increased density to HD in the FW or BW (Figure 1 C) induced slight degeneration of the intestinal mucosal lining. Signs of enterocyte degeneration, inflammatory cell infiltration in the lamina propria and abnormal thickness of intestinal wall, and congestion appeared (Figure 1 a, b, c).

Discussion

The fry of Nile tilapia is a critical life stage in the production cycle and requires special care (MacKinnon et al., 2023; Rakocy and Nair, 1984). Fish growth performance and welfare are affected strongly by many factors, such as water quality, nutritional value, and stocking density. Considering the importance of ensuring suitable conditions for the optimum growth performance of Nile tilapia fry, this study focused on two key factors that are a big concern in Egypt. More specifically, brackish water farming under high stocking density for Nile tilapia fry during the nursing stage.

The results indicated optimum levels for the pH and temperature but different significances for dissolved oxygen (DO), total ammonia, and water salinity in the aquaria used to grow Nile tilapia fry under different stocking densities for 60 days. These results indicate the proper management of the experimental conditions during the trial to keep the optimum conditions for the growth of Nile tilapia fry (MacKinnon et al., 2023). Fluctuations in water quality induce interruptions in the metabolic and physiological function and, thereby, the growth performance and survival rate (Mitra et al., 2023). However, in this study, no fluctuations were observed in the growth performance and survival rate of Nile tilapia fry due to the water salinity. Stocking density was only the significant factor in DO and total ammonia nitrogen (TAN) levels. Fry under high density (HD) suffer from low DO and high accumulated TAN, which may explain the reduction of the growth performance and survival rate either in freshwater (FW) or brackish water (BW). Overcrowding induces stressful conditions for fish and causes an interruption in feed intake, which accumulates in the water and results in high accumulated TAN (Cavatti Neto et al., 2023). Besides, the high number of fry consumes the DO, resulting in low available DO for the remaining fish (Manduca et al., 2021). Similarly, Luz et al. (2012) and Cavatti Neto et al. (2023) stated that growing Nile tilapia under high stocking density induced high levels of accumulated TAN and low DO.

In this study, growing Nile tilapia fry in FW or BW under LD showed the highest growth performance compared to middle density (MD) or HD. The significant reduction under MD or HD can be correlated with the increased levels of accumulated TAN involved in the increasing toxicity (Wu et al., 2018), thereby impairing fish's metabolic and physiological status (Abd El-Hack et al., 2022). Therefore, high TAN in the case of HD could be a direct reason for the growth performance reduction. In this regard, it has been reported that high stocking density led to high TAN accumulation in water leading to a high release of cortisol and glucose in the blood (Aketch et al., 2014; Onxayvieng et al., 2021; Wu et al., 2018). The high release of cortisol and glucose increases the energy cost and thereby deteriorates the physiological status of fish (Li et al., 2021). The survival rate of Nile tilapia fry under the current trial conditions showed a similar trend with the growth performance where the survival rate was impacted significantly with only the stocking density, while the water salinity had no marked effect. Fry in low density (LD) showed the highest survival rates either in FW or BW. These results indicate that high stocking density negatively impacted the health status of Nile tilapia fry, thereby lowering the stress resistance and increasing the mortality rate (Li et al., 2021). Similar observations were seen by Dawood et al. (2019) and Sarsangi Aliabad et al. (2022), who stated reduced survival rates in Nile tilapia cultured in high stocking density. Indeed, high stocking density is a chronic stressor involved in the deterioration of water quality, such as high ammonia accumulation and low DO, thereby reducing health status and increasing mortality (Li et al., 2021).

The results also illustrated that the feed intake (FI) and daily feed intake (DFI) were reduced while the feed conversion ratio (FCR) was increased by the effect of stocking density and not by the effect of water salinity. These results are in line with Abdel-Tawwab et al. (2014), who reported a reduction in feed utilization in Nile tilapia reared under high stocking density. The reduced feed utilization under the HD can also explain the poor growth performance of Nile tilapia fry reared in HD in FW or

BW. The feed utilization of fish is highly correlated with digestive enzyme activity and intestinal health (Dawood, 2021), which has been performed in this study to clarify the expected effects of water salinity combined with the stocking density factors. Digestive enzyme activities (protease, amylase, and lipase) were markedly reduced in the HD group under stocking density and water salinity effects. Similar results were reported by Zafar et al. (2022) and Liu et al. (2018), who indicated that the digestive enzyme activity was affected negatively by the high water salinity and stocking density in Nile tilapia. There is a strong relationship between intestinal health and the secretion of digestive enzymes to facilitate feed digestion and absorption (Dawood, 2021). On this occasion, the study investigated the effects of different stocking densities and water salinity on the morphological features of Nile tilapia fry intestines. The results showed that the intestine illuminated the normal structure of both intestinal mucosa and intestinal wall throughout the whole length of the middle intestine in the LD or MD under FW or BW. However, increased stocking density (HD) induced slight degeneration of the intestinal mucosal lining with marked signs of enterocyte degeneration, inflammatory cell infiltration in the lamina propria, abnormal thickness of the intestinal wall, and congestion. Impaired intestinal histological features in HD, regardless of the water salinity, can be associated with the oxidative stress induced by high stocking density (Wang et al., 2020). In this context, the high stocking density is known for the chronic stressful impact on fish, leading to the high release of free radicals (ROS) (Martos-Sitcha et al., 2020; Song et al., 2023). These free radicals cause lipid peroxidation and RNA breakdown and, thereby, failure of cell function (Song et al., 2023). Lipid peroxidation can be expressed by the malondialdehyde (MDA) level (Birnie-Gauvin et al., 2017), which showed high formation levels under the current study conditions in the case of Nile tilapia fry reared in MD or HD. Thus, impaired intestinal features can correlate to MDA's increased formation in fish grown in MD or HD. Consequently, the reduction of digestive enzyme activity and feed utilization in Nile tilapia fry in HD could be related to the impairment of intestinal health. Similarly, Refaey et al. (2018), reported impaired intestinal features in channel catfish (*Ictalurus punctatus*) grown in high stocking density.

Chronic stressful impacts resulting from the high stocking density are involved in reducing fish's antioxidative capacity (Wang et al., 2020), as illustrated in this study. Despite the water salinity effect, Nile tilapia fry in HD showed reduced superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Fish in FW or BW with LD or MD showed higher antioxidative responses than fish in HD, which can be explained by the increased MDA level. Stressful conditions activate oxidative stress and, thereby, ROS production involved in lipid peroxidation and MDA formation (Martos-Sitcha et al., 2020; Song et al., 2023). The high MDA formation activates the antioxidative enzymes to mitigate the impacts of ROS production (Winston, 1991). Similarly, Nile tilapia grown under high stocking density showed impaired antioxidative responses (Negm et al., 2021; Shourbela et al., 2021). Besides, high MDA formation may deteriorate the immune cells and cause malfunction, thereby the high possibility of infection with harmful invaders (Biller and Takahashi, 2018). In this regard, the study evaluated the lysozyme activity involved in the protection from infection with harmful microorganisms (Saurabh and Sahoo, 2008). The results indicated lowered lysozyme activity in the case of HD regardless of the water salinity. Similar results were reported by Liu et al. (2018) and Negm et al. (2021), who stated reduced lysozyme activity in Nile tilapia grown in high stocking density. The impaired lysozyme activity can be related to the high oxidative stress induced by high stocking density. As a result of this, the high mortality rate in Nile tilapia fry in HD groups can be correlated to weakened lysozyme activity and antioxidative capacity.

Conclusion

In conclusion, Nile tilapia fry can grow in brackish water (≈ 8 g saline/L) with 1000 to 2000 fry/m^3 without affecting the growth performance and feed utilization. The absence of negative impacts on the digestive enzyme activities and intestinal histological features has further confirmed these results. Besides, the stocking density of 1000 to 2000 fry/m^3 showed higher lysozyme activity and antioxidative responses than fry grown at high stocking density (4000 fry/m^3). The impaired immune and antioxidative responses are associated with the high release of malondialdehyde which may explain reduced growth performance and feed utilization under high stocking density. Therefore, stocking Nile tilapia fry at 1000 to 2000 fry/m^3 in brackish water (≈ 8 g saline/L) is possible with careful management of the water quality indices.

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