



Effect of a commercial probiotic on oxidative stress induced by high stocking density in Nile tilapia fingerlings

Zeinab I. Attia*, Hala A. Mostafa and Walaa M. Dawood

Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt

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Corresponding author:

Zeinab Ibrahim Attia ,
Zoology Department ,
Faculty of Science, Tanta
University, Egypt .

E-mail:

zainab.attia@science.tanta.edu.eg

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ABSTRACT

This study examined the effect of a commercial probiotic (*Lactobacillus acidophilus*) on oxidative stress induced by different levels of stocking density in Nile tilapia fingerlings. Fish were divided into 6 groups: group 1: control (90 fish/m³), group 2: control (90 fish/m³) with probiotic, group 3: high stocking density I (150 fish/m³), group 4: high stocking density I (150 fish/m³) with probiotic, group 5: high stocking density II (300 fish/m³), group 6: high stocking density II (300 fish/m³) with probiotic for 30 days. The results showed increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by increased stocking density compared with their respective control. Activities of AST and ALT at high stocking density I, II with addition of probiotic decreased compared to their respective high stocking densities in liver and white muscles. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST) decreased by increasing stocking density with compared with their respective control in liver and white muscle. However, they increased significantly in high stocking density I, II with probiotic compared with high stocking density I, II. The xanthine oxidase (XO) activity and malondialdehyde (MDA) level increased by increasing stocking density compared with their respective control and decreased in high stocking density I, II with addition of probiotic compared with high stocking density I, II. The data concluded that probiotics enhanced the oxidative stress caused by high stocking density in liver and white muscles in *Oreochromis niloticus*.

Keywords:

Antioxidant enzymes, liver, white muscles, high stocking density, probiotic, Nile tilapia

1. Introduction

Fish aquaculture may help supply the global demand for food. It is a rapidly expanding industry that offers consumers high-quality food. Additionally, it is essential for wellbeing and human health. (Amal *et al.*, 2021).

Nile tilapia (*Oreochromis niloticus*) is one of the most economically important farmed fish species produced in aquaculture systems worldwide (FAO, 2021). Its suitability for aquaculture is related to its ability to tolerate a wide range of environmental conditions. Its vast culture is attributed to its ability to feed on various food, rapid growth, popularity with consumers, ease of breeding in captivity, wide

availability to farmers and great source of high-quality protein and nutrients. Taking consideration of the optimal conditions for growth of Nile tilapia farming, lead to higher production (Vinicius *et al.*, 2020). These conditions are determined by factors including PH, temperature, photoperiod, dissolved oxygen (DO) and stocking density. Water quality is one of the most important factors for the culture of fish and should be constantly monitored in order to maintain the desirable characteristics for fish farming (Copatti and Amaral, 2009).

Stocking density is an important and critical factor which directly affects fish growth. High

stocking density increases competition for food and space intensifies among individuals, also affects fish feeding, physiological, metabolism, behavior, and immune function. High stocking density affects water quality by increasing fish faeces (like ammonia) and food wastes (Shalsabilla *et al.*, 2020).

Accumulation of waste due to high stocking density decreases the diffusion of oxygen, leading to a decrease in DO, decrease in oxygen absorption capacity which affects the metabolism of the fish, so the growth rate decreased. High stocking density causes crowding stress, triggering oxidative stress which increases in reactive oxygen species (ROS) that causes oxidant/ antioxidant imbalance (Ahmed *et al.*, 2000).

2. Materials and Methods

Fish Husbandry

Fingerlings of tilapia fish were obtained from the fish hatching pond in Fowa City (Kafr El-Sheikh Governorate, Egypt). Fish were transferred in well-oxygenated cellophane gas bags then distributed in thirty glass aquaria with a capacity of 36 liters per aquarium. Its dimensions equal to (40 l x 30 W x 30 H) cm, respectively 5 fish per aquarium (90 fish/m³) in control, 7 fish per aquarium (150 fish/m³), in high stocking density I and 10 fish per aquarium (220 fish/m³), in high stocking density II. Each aquarium was equipped with an air pump. Fish were acclimated to laboratory conditions at room temperature. The entire acclimation period was 4 weeks; taking into consideration the optimum temperature during all experiments was 26°C. Water of aquaria was exchanged using dechlorinated water. Fish were exposed to a normal light dark cycle (12 l: 12D) during the experiment and were fed daily a standard diet (30% protein with total energy 4000 kcal kg⁻¹) (Hand Man Animal Nutrition Company, Egypt).

Probiotic

A commercial probiotic (*Lactobacillus acidophilus* 2 × 10⁸ CFU/gm) composed of: *Bacillus subtilis* fermentation extract 100 grams, 50 grams: Xylanase 1250 unit, Hemicellulase 2750 unit, Beta - glucanase 2250 unit; *Aserigulus Oryzae* fermentation Extracts

Oxidative stress is affected by extrinsic and intrinsic factors in fish. High stocking density leads to oxidative stress in fish (Taheri *et al.*, 2018). Fish have antioxidant enzymes to protect them against oxidative conditions.

Probiotics have been defined as "microbial cells that are administered in such a way to enter the gastrointestinal tract and to be kept alive, with the aim of improving health". Probiotics are one of the alternatives that have been widely used in the aquaculture industry (Elsabagh *et al.*, 2018). The aim of this study is to assess the ameliorating effect of probiotics on the oxidative stress results from high stocking density.

50 grams: Alpha Amylase 2.5×10³-unit, Protease 12500 unit; Cellulase 4500 unit; Dextrose as a carrier up to 1 kg (Micro-BACLA).

Experimental Design

Fish were divided into 6 groups, each group composed of five aquaria. Group 1: control 90 fish/m³ (5 fish 10 g ± 2). Group 2: group 2: Positive control 90 fish/m³ (5 fish 10 g ± 2) with addition of probiotic, group 3: high stocking density I 150 fish/m³ (7 fish 10 g ± 2). group 4: high stocking density I 150/m³ fish (7 fish 10 g ± 2) with addition of probiotic., group 5: high stocking density II 300 fish/m³ (14 fish 10 g ± 2) group 6: high stocking density II (300 fish/m³) with addition of probiotic. In the experiment, fish reared for 30 days.

Tissue Sampling

The liver and white muscles were carefully excised, avoiding squeezing the tissue, then washed in ice-cold isotonic NaCl saline, blotted dry and weighed. The liver and white muscles were homogenized in ice-cold phosphate buffer saline (PBS) (50 mM, pH 7.4) 10% (w/v) using Omni international homogenizer (USA) at 2.2×10³ rpm for 20 s each with 10 s intervals. Activity of AST and ALT were determined by IFCC method (Carl & Edward, 2008) at wavelength 340 nm Kinetic. U.V.

Antioxidant enzyme activities and biochemical markers assays were measured by automated ELISA System using (Chemwell 2099, Gama Trade Company).

Statistical analysis

Data were presented as means \pm standard deviation (SD). The statistical evaluation of all

data was done using one-way analysis of variance (ANOVA) followed by Sidak test

3. Results

The obtained results of growth rate represented in table1, liver and white muscles enzymes (AST, ALT) represented in tables 2 and 3 and specific antioxidant enzymes activities and oxidative stress biomarkers in Nile tilapia liver and white muscles were represented in tables 4-7. The growth rate decreased significantly with increasing stocking density, on other hand the growth rate improved with additional probiotic. The activity of AST and ALT in liver and white muscles were significantly increased by increasing stocking density in comparison with control, on other hand their activities were significantly decreased in high stocking density with additional probiotic in comparison with high stocking density. The activity of SOD,

CAT, GPX, GR and GST were significantly decreased by increasing stocking densities compared with their respective control in liver and white muscles. The activities of SOD, CAT, GPX, GR and GST were significantly increased in high stocking density with additional probiotic compared with their respective high stocking density groups in liver and white muscles. The activities of XO and MDA were significantly increased by increasing stocking density compared with control in liver and white muscles. The activities of XO and MDA and were significantly decreased in high stocking density with additional probiotic compared with their respective high stocking density groups.

Table 1. Effect of different stocking densities and probiotic on growth rate of Nile tilapia fingerlings.

	Control	C.Pro.	H.SD.(1)	H.SD. (1) Pro.	H.SD. (2)	H.SD.(2). Pro
Initial weight (Mean \pm SD)	10.56 \pm 4.0	12.30 \pm 3.5	10.20 \pm 3.0	10.0 \pm 4.0	10.2 \pm 3.0	10.40 \pm 5.0
Final weight (Mean \pm SD)	20.56 \pm 4.0 *	26.6 \pm 7.0 *	17.38 \pm 3.0*	18.8 \pm 3.0*	15.62 \pm 1.5 *	18.60 \pm 4.0 *
Absolute growth rate (gr/ day)	0.33	0.47*	0.23*	0.29	0.18*	0.27
Relative growth rate (%n/30 day)	94.69	116*	70.3*	88	53*	78
Specific growth rate (%n/ day)	2.22	3.19*	1.77	2.1	1.4*	1.9

Control: 90 fish /m³; C.Pro: Control 90 fish /m³ with probiotics; H.SD.(1): 150 fish/m³; H.SD. (1)Pro: 150 fish/m³ with probiotics; H.SD.(2): 220 fish m³; H.SD.(2): 300 fish /m³; H.SD.(2)Pro: 300 fish /m³ with probiotics. Each reading represents Mean \pm SD of n = 10 *: Significant vs. control at $p < 0.05$

Table2. Effect of different stocking densities and probiotic on AST, ALT activities (μ g/Min/gram wet weight tissue) in liver.

	Control	C.Pro.	H.SD.(1)	H.SD. (1) Pro.	H.SD. (2)	H.SD.(2). Pro
AST	24.2 \pm 1.8	23.9 \pm 2.6	34.8 \pm 2.8*	29.2 \pm 3.1	13.14 \pm 3.2*	16.02 \pm 4.9*
ALT	17.6 \pm 5.6	15.96 \pm 4.7	26.0 \pm 3.8*	17.2 \pm 3.7*	50.06 \pm 5.38*	37.1 \pm 4.4*

Control: 90 fish /m³; C.Pro: Control 90 fish /m³ with probiotics; H.SD.(1): 150 fish/m³; H.SD. (1)Pro: 150 fish/m³ with probiotics; H.SD.(2): 220 fish m³; H.SD.(2): 300 fish /m³; H.SD.(2)Pro: 300 fish /m³ with probiotics. Each reading represents Mean \pm SD of n = 10 *: Significant vs. control at $p < 0.05$

Table3. Effect of different stocking densities and probiotic on AST, ALT activities ($\mu\text{g}/\text{Min}/\text{gram}$ wet weight tissue) in white muscles.

	Control	C.Pro.	H.SD.(1)	H.SD. (1) Pro.	H.SD. (2)	H.SD.(2). Pro
AST	21.6 \pm 5.5	22.5 \pm 5.1	30.0 \pm 2.7	23.5 \pm 2.8	55.8 \pm 10.09*	39.4 \pm 7.7*
ALT	23.1 \pm 2.3	28.8 \pm 5.02	33.8 \pm 3.9*	21.8 \pm 3.1*	36.5 \pm 6.2*	22.9 \pm 3.4*

Control: 90 fish /m³; C.Pro: Control 90 fish /m³ with probiotics; H.SD.(1): 150 fish/m³; H.SD. (1)Pro: 150 fish/m³ with probiotics; H.SD.(2): 220 fish m³; H.SD.(2): 300 fish /m³; H.SD.(2)Pro: 300 fish /m³ with probiotics. Each reading represents Mean \pm SD of n = 10 *: Significant vs. control at $p < 0.05$

Table 4. Effect of different stocking densities and probiotic on activities of antioxidant enzymes ($\mu\text{M}/\text{min}/\text{gram}$ wet weight tissue), except that SOD activity (U/mg wet weight tissue) in liver.

	Control	C.Pro.	H.SD.(1)	H.SD. (1) Pro.	H.SD. (2)	H.SD.(2). Pro
SOD	23.18 \pm 3.0	25.5 \pm 3.0	15.02 \pm 0.9*	16.1 \pm 3.5	8.8 \pm 1.64*	16.98 \pm 1.9*
CAT	25.26 \pm 1.09	27.3 \pm 2.1	19.2 \pm 1.7*	22.5 \pm 1.8	12.98 \pm 2.2*	23.58 \pm 2.1*
GPX	12.88 \pm 1.5	14.8 \pm 1.8	10.6 \pm 1.4*	13.4 \pm 1.2*	9.42 \pm 1.36*	12.48 \pm 0.8*
GR	5.42 \pm 1.1	5.2 \pm 0.8	3.4 \pm 0.8*	3.6 \pm 0.5	3.32 \pm 1.56*	4.02 \pm 0.93*
GST	23.3 \pm 3.06	24.1 \pm 3.5	17.0 \pm 1.6*	21.8 \pm 1.6*	13.8 \pm 2.4*	18.8 \pm 1.8*
XO	4.22 \pm 1.4	5.96 \pm 1.6	12.2 \pm 1.5*	7.2 \pm 0.9*	19.6 \pm 1.6*	11.6 \pm 2.1*
MDA	12.6 \pm 1.8	13.1 \pm 2.6	17.2 \pm 1.9*	13.8 \pm 1.9	18.7 \pm 1.78*	14.1 \pm 3.1

Control: 90 fish /m³; C.Pro: Control 90 fish /m³ with probiotics; H.SD.(1): 150 fish/m³; H.SD. (1)Pro: 150 fish/m³ with probiotics; H.SD.(2): 220 fish m³; H.SD.(2): 300 fish /m³; H.SD.(2)Pro: 300 fish /m³ with probiotics. Each reading represents Mean \pm SD of n = 10 *: Significant vs. control at $p < 0.05$

Table5. Effect of different stocking densities and probiotic on activities of antioxidant enzymes ($\mu\text{M}/\text{min}/\text{gram}$ wet weight tissue), except that SOD activity (U/mg wet weight tissue) in white muscles.

	Control	C.Pro.	H.SD.(1)	H.SD. (1) Pro.	H.SD. (2)	H.SD.(2). Pro
SOD	29.0 \pm 2.0	28.6 \pm 2.0	17.8 \pm 1.5*	22.6 \pm 2.1*	13.7 \pm 1.8*	18.4 \pm 2.1*
CAT	27.9 \pm 2.5	29.0 \pm 2.0	15.7 \pm 1.6*	18.3 \pm 1.0	11.3 \pm 1.4*	15.7 \pm 0.7*
GPX	14.8 \pm 1.2	13.9 \pm 1.1	10.6 \pm 1.4*	13.7 \pm 1.2*	8.0 \pm 2.2*	12.5 \pm 1.6*
GR	4.2 \pm 1.2	5.7 \pm 1.9	1.5 \pm 0.7*	2.9 \pm 0.5	1.1 \pm 0.7*	2.3 \pm 0.7
GST	23.3 \pm 2.7	25.5 \pm 3.3	17.7 \pm 1.5*	22.3 \pm 1.5*	13.5 \pm 2.4*	18.8 \pm 1.5*
XO	4.7 \pm 0.8	5.4 \pm 1.4	13.9 \pm 1.2*	14.2 \pm 3.6	24.8 \pm 4.0*	13.9 \pm 1.98*
MDA	4.9 \pm 1.05	3.4 \pm 1.17	19.0 \pm 2.2*	13.0 \pm 3.1*	26.3 \pm 2.4*	11.4 \pm 1.29*

Control: 90 fish /m³; C.Pro: Control 90 fish /m³ with probiotics; H.SD.(1): 150 fish/m³; H.SD. (1)Pro: 150 fish/m³ with probiotics; H.SD.(2): 220 fish m³; H.SD.(2): 300 fish /m³; H.SD.(2)Pro: 300 fish /m³ with probiotics. Each reading represents Mean \pm SD of n = 10 *: Significant vs. control at $p < 0.05$

4. Discussion

This study conducted with growth rate, liver and white muscles enzymes (AST and ALT) in Nile tilapia fingerlings. In addition to oxidative stress biomarkers and antioxidant defense in liver and white muscles. To analyze the effects of various high stocking densities and the function of the probiotic, it was crucial to evaluate the activity of antioxidant enzymes and lipid peroxidation in high stocking density (I), high stocking density (II), high stocking

density (I) with probiotics and high stocking density (II) with probiotics in the liver and white muscles. Tilapia cultivated at various stocking densities had different average growth rates. The results showed low growth rate in high stocking density (II) compared with their respective control. Growth rate of high stocking density (I) was not affected, and fish showed final weight as a control. indicating that stronger fish social interaction may not alter fish feeding behaviour, proving tilapia ability to tolerate range of stocking density. These findings are consistent with Yao *et al.*, 2022,

which found that *M. pellegrini* weight gain was significantly lower at higher stocking density groups than in relatively lower ones, indicating that growth was adversely impacted when density exceeded a particular value for these fish species.

According to this study, which agrees with Waleed *et al.*, 2018, juvenile tilapia stocked in high density experienced a drop-in growth rate because of crowding or because of physiological reactions linked to stress. Which emphasized the negative effect of high stocking density. However, there was an increase in growth of groups receiving probiotics compared to respective controls, demonstrating the improving effect of probiotics. It was evident since the weight significantly increased in the control group.

Which agrees with Mary *et al.*, 2019 as they suggested that addition of probiotics improved growth rate in high stocking density groups compared with high stocking density groups with out of probiotic groups. The present study results go in parallel with the study of Merrifield *et al.*, 2010 who stated that probiotics improved digestion of food by producing digestive enzymes or alterations of the gut environment, translated to better growth.

was adversely impacted when density exceeded as amino acid-metabolizing enzymes, AST is mainly present in cardiomyocytes and hepatocytes, while ALT mainly exists in the liver. Increased AST and ALT activities may reflect an excess of amino acid hydrocarbons being used to meet the higher energy demand. This shows that confinement stress results in a larger mobilization of free amino acids, which in turn created glucose to handle the stress through a stronger gluconeogenesis pathway.

our This study goes in parallel with Soheila *et al.*, 2023 stated, higher stocking density caused higher plasma AST and ALT as the stress response in tilapia, and they also concluded that stocking density of tilapia up to 150 fish m⁻³ showed an adverse effect on the liver functions ALT activity were significantly higher in the group of 500 fish/m³ than the groups of 125 and 250 and confirmed that the plasma AST and ALT was significantly increased at high stocking density at the end of

the experiment. This degree of response was caused by the influence of stocking density as a stress factor which suggested that increased stocking density caused stress to fish.

Soltan & El-Laithy, 2008 noticed that tilapia treated diets with probiotic had significantly decreased AST and ALT values compared to control group. Which agrees with our result and another study where AST and ALT levels significantly decreased when tilapia fed diets supplemented with probiotic compared to control group.

Usually, antioxidant enzymes are upregulated by elevated levels of ROS. However, not all fish showed an overall increase in key markers of redox-activated antioxidant defense in response to elevated high stocking-induced oxidative stress Changes in the activities of antioxidant enzymes or ROS production during high stocking density are species and tissue specific. stress (Leveelahti *et al.*, 2014). Changed in the activities of antioxidant enzymes or ROS production during high stocking density are species and tissue specific (Lau *et al.*, 2019).

There is a decrease in activities of SOD, CAT, GR, GPX and GST by increasing stocking density in liver and white muscles compared with control related to stress which can activate the intracellular production of ROS. High ROS can exhaust the antioxidant defense system thus reducing the enzyme antioxidant activities.

Fish groups stocked at a high rate suffered from oxidative stress compared to respective control. The present research in agreement with a samar *et al.*, 2021 which indicated that high stocking density had significantly reduced the tilapia serum levels of SOD. This could be considered a response to the nonstop stresses of high stocking density and might mirror the restricted capacity of the antioxidant systems in tilapia.

SOD catalyzes the dismutation of superoxide anions to hydrogen peroxide, which is subsequently detoxified to oxygen and water by Catalase (Zhang *et al.*, 2007). Antioxidant activity of SOD and CAT, appear to have an important role in computing the generation of superoxide radical and hydrogen peroxide from the intense metabolic activity characteristic of these tissues.

The first line of antioxidant defense in fish are SOD, CAT, and GPx, which convert superoxide ion to oxygen and water through sequential reactions (Seyyed *et al.*, 2019).

our finding was inconsistent with El Hawwary *et al.*, 2018 in the reduction of SOD and CAT activities by increasing stocking density. Also agrees with a Xin *et al.*, 2022 which showed that intestinal SOD and CAT enzyme activities significantly decreased as the stocking density increased. Conversely, increased levels of antioxidants were also observed in fish under high density, suggesting a positive response of the antioxidant defense system to stress may also happen in another fish species (Yao *et al.*, 2022) which showed that intestinal SOD and CAT enzyme activities significantly decreased as the stocking density increased. Conversely, increased levels of antioxidants were also observed in fish under high density, suggesting a positive response of the antioxidant defense system to stress may also happen in another fish species (Yao *et al.*, 2022)

In our study we observed a positive effect of probiotic on activities of antioxidant enzymes (SOD, CAT, GR, GPX and GST), where their activities were clearly decreased in high stocking density groups after 30 days compared to control. Emmanuel *et al.*, 2018 studied fish fed with probiotic and evaluation of SOD activity in all treated groups which showed variable levels at both time points but with higher SOD activity compared with the untreated group after 4 weeks compared to control group. All treated groups demonstrated a significant increase in CAT activities after 2 and 4 weeks. The present study also agrees with Gehan *et al.*, 2020 study which showed that dietary supplementation of probiotic improved the antioxidant status SOD and CAT activities of the fish which suggest improved immunity and health status in Nile Tilapia.

In this experiment study GPX activity in liver and white muscles which was significantly decreased by increasing stocking density compared to control. Which agrees with a Taylor & Zenteno *et al.*, 2011 study on Pacific white shrimp (*Litopenaeus vannamei*) stated that activity of the antioxidant enzyme GPx in extracts of muscle and hepatopancreas from white shrimp increased in case of probiotic.

Xanthine oxidase levels increased in fish with high stocking density in comparison with control. This agrees with Hegazy *et al.*, 2010 study where hepatic XO activity was higher in fish exposed to oxidative stress. Demonstrated that this enzyme is the main pathway related to ROS production and consequent damage during stress exposure. Beedham, 2002 studies on XO showed that modulation of enzymes activity, co factors availability, substrate concentration and oxygen tension all affect rates of intracellular ROS production. Although XO generates ROS enzyme exists predominantly as a dehydrogenase, reacting with NAD^+ . In the present study XO levels decreased in different stocking density with the addition probiotic compared to their respective high stocking density.

The increase in MDA level is due to the occurrence of oxidative stress which related to imbalance between ROS and the ability of the biological system to detoxify readily the reactive intermediates. Stress leading to the formation of various active compounds including high level of MDA (Zorawar *et al.*, 2014). Fish reared in high stocking density had significantly higher MDA level. Which is confirming that high stocking density as a stressor can stimulate the formation of ROS in fish (Wang *et al.*, 2019). In fish, increased lipid peroxidation has always attracted widespread attention under high stocking densities. Zhou *et al.*, 2020 found that the high stocking density promoted MDA formation in the liver, blood, or intestine of fish. Excess ROS may react with unsaturated fatty acids on cell membranes, inducing lipid peroxidation. It has been reported that a large number of harmful products from lipid peroxidation are capable of inactivating many cellular proteins, inducing inflammation and damaging cells or tissues. Similarly, the formation of ROS in fish (Wang *et al.*, 2019). increased MDA level was also observed in the plasma and liver of *M. Salmoides*, indicating that the high density induced lipid peroxidation after 90 days of farming the rice fish farming system (Zaki *et al.*, 2020).

There was significance decrease in MDA case of additional probiotic in comparison with high stocking density.

Conclusion:

High stocking density led to oxidative stress in Nile tilapia liver and white muscles at different levels of stocking density which led to a decline in the activity of antioxidant enzymes.

6. References

- Ahmad I, Hamid T, Fatima M, Chand H, Jain S, Athar M, Raisuddin S, 2000. Induction of hepatic antioxidants in freshwater catfish (*Channa punctatus* Bloch) is a biomarker of paper mill effluent exposure. *Biochim. Biophys. Acta. (BBA)*, 1523 (1): 37–48.
- Amal M, Soaad A, Amaal M, 2021. Accumulation of heavy metals in tilapia fish species and related histopathological changes in muscles, gills and liver of *Oreochromis niloticus* occurring in the area of Qahr El-Bahr, Lake Al-Manzalah. *Egypt. Oceanol. Hydrobiol. Stud.* 50 (1):1 - 15.
- Beedham C, Panoutsopoulos G, 2002. Enzymatic oxidation of vanillin, isovanillin and protocatechuic aldehyde with freshly prepared Guinea pig liver slices. *Cell. Physiol. Biochem.* 15: 089-098.
- Catherine R, Seth K, Ruby A, Emmanuel T, Sena A, Mathew O, 2022. Accelerating pond aquaculture development and resilience beyond COVID: Ensuring food and jobs in Ghana. *Aquaculture.* 547: 73-74.
- El-Hawarry W, Mohamed R, Ibrahim S, 2018. Collaborating effects of rearing density and oregano oil supplementation on growth, behavioral and stress response of Nile tilapia (*Oreochromis niloticus*). *Egypt. J. Aquat. Res.*44: 173–178.
- Elsabagh M, Mohamed R, Moustafa E, Hamza A, Farrag F, Decamp O, Dawood M, Eltholth M, 2018. Assessing the impact of *Bacillus* strains mixture probiotic on water quality, growth performance, blood profile and intestinal morphology of Nile tilapia, *Oreochromis niloticus*. *Aquac. Nutr.* 24: 1613–1622.
- Emmanuel D, Vivian H, Felix K, Gyamfua A, Yishan L, Shuyan C, Melody A, 2018. The use of *Bacillus* species in maintenance of water quality in aquaculture. *Aquac. Reports.* 18: 7340-7352.
- Probiotic acts to compensate for this reduction in these enzymes' activity in high stocking densities. The changes in antioxidant enzymes activities in response to high stocking density may be crucial for avoiding oxidative damage and maintaining the quality of farmed fish.
- FAO, IFAD, UNICEF, WFP, WHO, 2021. The State of Food Security and Nutrition in the World.
- Gehan I, El-keredy M, Abeer M, Nehal A, Naena and Elgohary M, 2020. Using of some phytobioyics and probiotics as promotors to cultured Nile Tilapia. *Int. J. Fish. Aquat. Stud.*8: 148-159.
- Hegazy M, Attia Z, Ashour o, 2010. Oxidative stress and antioxidant enzymes in liver and white muscle of Nile tilapia juveniles in chronic ammonia exposure. *Aquatic toxicol.* 99: 118 – 125.
- enzymes, immunity, and oxidative status of Nile tilapia (*Oreochromis niloticus*) reared in intensive conditions. *Slov. Vet. Res.* 56 (22): 99–108.
- Mary A, Opiyoa b, James J, Charles C, Ngugi C, Harrison C, Karisa d, 2019. Different levels of probiotics affect growth, survival and body composition of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Sci. Afr.*4: 2468-2276.
- Merrifield D, Bradley G, Baker R, Davies S, Dimitroglou A, Bradley G, Baker R, Davies S, 2010. Probiotic applications for rainbow trout (*Oncorhynchus mykiss*, *Walbaum*). Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. *Aquac. Nutr.* 16: 504–510.
- Sahin K, Yazlak H, Orhan C, Tuzcu M, Akdemir F, Sahin N, 2014. The effect of lycopene on antioxidant status in rainbow trout (*Oncorhynchus mykiss*) reared under high stocking density. *Aquaculture.* 418: 132–138.
- Samar S, Nahla E, Amany I, Amel M, Mohammed A, 2021. The efficiency of dietary *Sargassum aquifolium* on the performance, innate immune responses, antioxidant activity, and intestinal microbiota of Nile Tilapia (*Oreochromis niloticus*) raised at high stocking density. *J. Appl. Phycol.* 33: 4067–4082.
- Seyyed M, Morteza Y, 2019. Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response,

- immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.* 25: 298-309.
- Shalsabilla N, Ayi Y, Iis R, Ibnu B, Bioshina S, 2020. Effect of Stocking Density on Water Quality of Tilapia (*Oreochromis niloticus* Linnaeus, 1758) in Round Container with Current and Venturi Aeration System. *World Sci. News.* 145: 397-408.
- Soheila N, Hamid M, 2023. Optimizing stocking density in biofloc culture of juvenile common carp using growth and immune-biochemical indices as indicator. *Aquac. Studies.* 23: 12-23.
- Soltan M, El-laithy E, Fouad I, Elfeky A, 2008. Growth and feed utilization of Nile tilapia, *Oreochromis niloticus* fed diets containing probiotic. *Glob. Vet.* 17: 442–450.
- Taheri M, Peymen Y, Mehdi S, Hamed P, Seyyed M, 2018. Dietary sodium butyrate (Butirex C4) supplementation modulates intestinal transcriptomic responses and augments disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.* 92: 621- 628.
- Taylor P, Delia P, Zenteno S, 2011. Antioxidant enzyme activities in Pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation. *Aquac.* 318: 379-383.
- Wang Y, Xu G, 2019. Effect of stocking density on growth, serum biochemical parameters, digestive enzymes activity and antioxidant status of largemouth bass, *Micropterus salmoides*. *Pakistan J. Zool.* 51: 1509–1517.
- Xin W, Qi Y, Zhi-xin G, 2022. Effects of different stocking densities on the growth performance and antioxidant capacity of Chinese mitten crab (*Eriocheir sinensis*) in rice crab culture system. *Aquac. Int.* 30: 883 – 898.
- Yao W, Zhi Z, Yuejia S, Qing J, Yujie G, 2022. Influence of Stocking Density on Growth Performance, Intestinal Antioxidant Status, and Immune-Related Gene Expression of Endangered *Megalobrama pellegrini* Reared in a Flow-Through System. *N. Am. J. Aquac.* 84: 172–180.
- Yuan J., Meng N., Mei L., Jianfeng L., Guoqiang M., Zhimin G., (2021). Stocking density alters growth performance, serum biochemistry, digestive enzymes, immune response, and muscle quality of largemouth bass (*Micropterus salmoides*) in in-pond raceway system. *Fish. Physiol. Biochem.* 47: 1243–1255.
- Zaki M A, Alabssawy AN, Nour AM, And Abdel Daim M, 2020. The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions. *Aquac. Rep.* 16: 100282.
- Zhang L, Fan Q, Zhao Z, Xiong D, 2007. The effects of chronic crowding stress on growth and blood biochemical indexes in Carp. *J. Dalian Med. Univ.* 22: 465-469.
- Zhou L, Zhang J, Yan M, Tang S, Wang X, Qin J, Chen L, Li E, 2020. Inulin alleviates hypersaline-stress induced oxidative stress and dysbiosis of gut microbiota in Nile tilapia (*Oreochromis niloticus*). *Aquac.* 529: 735681.
- Zorawar S, Mohamed Y, Samina F, Sheik S, 2014. Plant derived polyphenols, other drugs targeting NF-Kb and therapeutic implications. *Br. Biomed. Bull.* 2: 422-430.