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Efficiency of Dietary *Bacillus subtilis* on Growth Performance, Hematology, and Gut Histology of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The present investigation assessed the impact of nutritional enrichment with *Bacillus subtilis* on growth performance, hematological indices, physiological biomarkers, and intestinal histology of the Nile tilapia (*Oreochromis niloticus*). A total of 120 Nile tilapia (115 ± 0.01 g) were indiscriminately allocated to four dietary groups and were fed experimental diets containing 0.0 (control), 0.5, 1.0, and 2.0g/ kg of *B. subtilis* for two months. Fish given 2.0g *B. subtilis* /kg diet showed a substantial improvement ($P< 0.05$) in growth indicators, such as final weight, weight gain, specific growth rate, and feed conversion ratio. Comparing the 1.0 and 2.0g/ kg groups to the control, haematological data revealed a substantial $(P< 0.05)$ boost in the count of red blood cells, hemoglobin concentration, and haematocrit. Biochemical analysis revealed elevated total protein and albumin concentration in the Nile tilapia given higher probiotic levels. Histological examination of the anterior intestine showed improved villus height and goblet cell intensification, while liver sections exhibited no pathological alterations. The outcomes of the current assessment concluded that dietary insertion of *B. subtilis* at 2.0g/ kg enhances growth, wellbeing, hemato-biochemical condition, and intestinal health of *O. niloticus*, suggesting its potential role as a functional probiotic addition in aquaculture.

INTRODUCTION

Over the last ten years, aquaculture has become the most rapidly expanding segment of global animal-based food production **(FAO, 2024)**. It currently supplies around half of the world's seafood, making it essential for supplying the increasing need for animal-derived protein in many nations **(Jahangiri & Esteban, 2018)**. Among farmed species, the Nile tilapia (*Oreochromis niloticus*) holds significant economic value and is extensively cultivated worldwide **(Mahi *et al.*, 2022)**. With the continuous growth of the global population, the demand for food, especially for cultivated aquatic species, has increased

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substantially **(Sarker *et al.,* 2022)**. *O. niloticus*, the backbone of Egypt's aquaculture industry, accounts for 61% of total output capacity and 44% of production value **(Walakira *et al.,* 2023)**.

Antibiotics have traditionally been used extensively to avert infections in fish, prawns, and crab aquaculture. Nevertheless, their prolonged application has become unsustainable and less effective owing to the rise of antibiotic-resistant microbes **(Ayisi *et al*., 2017)**. Consequently, the aquaculture sector has progressively adopted probiotics as a viable alternative to synthetic antibiotics and chemical therapeutics. This transition is driven by accumulating evidence indicating that probiotics can promote fish growth, enhance tolerance to stress, regulate immunity, and improve defenses against infectious illnesses **(Sumon *et al.*, 2022)**.

Probiotics are characterized as "living bacteria provided at a sufficient quantity that confer advantageous impacts on host wellness and immune functions" **(Ghosh *et al.,* 2023)**. The global aquaculture sector has increasingly embraced the concept of antibiotic-free farming. Within this framework, alternatives such as prebiotics and probiotics have garnered significant interest due to their potential to promote fish health, mitigate stress-induced effects, and reduce the occurrence of infectious diseases across a range of species **(Naiel *et al.,* 2022)**.

Several studies have reported the advantageous influence of *Saccharomyces cerevisiae, Lactobacillus acidophilus, Streptococcus faecium,* and *Bacillus subtilis* on the performance of *O. niloticus* **(El-Saadony *et al.,* 2021)**. These probiotic strains have been associated with enhanced growth rates, improved digestive function, strengthened immune responses, and increased resilience against both biological and environmental stressors **(Yilmaz *et al.,* 2022)**. The application of probiotics in aquaculture is regarded as an alternative immunoprophylactic approach, offering a complementary or substitute method to traditional vaccines and pharmaceutical interventions **(Naiel *et al.,* 2021)**.

In this context, the current investigation intended to assess the impact of varying dietary *B. subtilis* on the growth efficiency, hematological, physiological, and intestinal histology of *O. niloticus*. The outcomes of the current research are expected to provide significant insights into the use of probiotics as an efficient feed supplement in sustainable aquaculture systems.

MATERIALS AND METHODS

Trial location and fish source

The research study was applied at the Fish Farming and Technology Institute, Suez Canal University, from 2 October to 29 December, 2022, spanning 60 days. The Nile tilapia (*Oreochromis niloticus)* was obtained from the Fish Farming and Technology Institute. A total of 120 fish were stocked in two cement ponds for 15 days to allow acclimatization.

During acclimation, the Nile tilapia was given a commercial feed containing 32% crude protein.

Experimental design and diets

Four isonitrogenous feeds were formulated by enriching a commercial feed with graded levels of a probiotic (Aqua Grow®, Canal Aqua Cure, Port Said, Egypt) preparation containing *B. subtilis* (2 × 10 11 cell/ g) at 0.0 (control), 0.5, 1.0, and 2.0g/ kg, contributed to treatment numbers T1, T2, T3, and T4, respectively. All diets were weighed, dried (using air drying), and then allocated in plastic bags for storage in a fridge at 4°C pending usages.

Fish were stocked separately at 10 fish/tank for a total of 12 tanks (1 m^3 each), representing four experimental treatments with three replicates. Fish were hand-fed the trial diets three times daily at 9:00 am, 12:00 pm, and 2:00 pm until they appeared to be satisfied. Fish in all treatment tanks were provided with freshwater and aerated by an air stone in each tank. Fish weights were assessed biweekly.

Water quality monitoring

The water temperature was set at 28°C in the tanks by using central heaters, while pH and dissolved oxygen levels were measured via a portable multi-parameter instrument. Total ammonia nitrogen was assessed weekly colorimetrically via kits from (sera test).

Growth variables and feed efficiency

Growth and feed efficiency were assessed using the following equations, as described by **Carlos (1988)**:

•**Weight gain (WG, g)** = final weight – initial weight

•**Specific growth rate (SGR, % /day)** = [(ln final weight – ln initial weight) / 60 (trial period)] × 100

•**Feed conversion ratio (FCR)** = Feed intake / Weight gain

•**Average Daily Gain (ADG, g /day**) = Weight gain (WG, g) / trial period (60 days).

Somatic indices

The hepatosomatic index (HSI) was determined to assess liver development as a proportion of total body weight, via the subsequent equation according to **Munkittrick and Dixon (1988):**

HSI = (weight of Liver / Total body weight) × 100

Haematological parameters

Blood specimens were obtained from the caudal vein of the Nile tilapia, with three individuals sampled per tank for each trial group. Prior to sampling, fish were sedated using

clove oil at a concentration of 0.5mL/ L. Ethylenediaminetetraacetic acid (EDTA) was used as an anticoagulant. Plasma was subsequently separated by centrifugation and was preserved for further hematological study.

Complete blood count (CBC) parameters were evaluated for all treatments. Red blood cell (RBC) counts were performed using a hemocytometer with EDTA-treated blood samples, and results were expressed as $\times 10^6$ cells μL^{-1}. Hematocrit (Hct) values were measured by centrifuging heparinized microcapillary tubes at 5000 rpm for 5 minutes. Hemoglobin (Hb) levels were measured calorimetrically utilizing Boehringer Mannheim diagnostic kits, according to the approaches mentioned by **Britton (1963)** and **Dacie and Lewis (1991)**.

Erythrocyte indices were calculated using the subsequent formulas:

•Mean corpuscular volume (MCV) = (Hct × 10) / RBC ($\times 10^6$ μL^{-1})

•Mean corpuscular hemoglobin (MCH) = (Hb × 10) / RBC ($\times 10^6$ μL^{-1})

•Mean corpuscular hemoglobin concentration (MCHC) = (Hb × 100) / Hct

Blood biochemical analysis

The plasma obtained by centrifuging blood specimens taken with an anticoagulant at 5000 rpm for 15 minutes was frozen at -20°C pending further examination.

•Total protein: Measured by the biuret method **(Henry, 1974)**.

•Albumin: Assayed using a bromocresol green dye-binding method **(Busher, 1990)**.

•Globulin: Calculated as total protein minus albumin.

•Total lipid: Quantified by the sulfuric acid–vanillin method **(Schmit, 1964)**.

•Urea: Measured using the urease-GLDH method **(Trinder, 1969)**.

Liver enzyme assays

The concentrations of aspartate (AST) and alanine (ALT) aminotransferase were assessed using commercially available diagnostic kits (from Spectrum). Absorbance was recorded at 546nm, and enzyme activities were calculated based on the manufacturer's guidelines.

Histological examination

Liver and intestinal specimens were gathered from five Nile tilapia from each trial group. The tissues were carefully excised and stored in 10% formalin, then dehydrated using a gradient of ethanol concentrations and clarified in xylene. Following fixation, the specimens were submerged in paraffin wax. A Euromex Holland microtome (Arnhem, The Netherlands) was then used to create slices with a diameter of 5µm. Tissue sections were

dyed with Harris hematoxylin and eosin (H&E) and were then viewed under a light microscope. Through the utilization of a microscope-mounted camera, photomicrographs were acquired.

Statistical investigation

Using the statistical program SPSS (version 26.0), the data were analyzed. To determine whether there were statistically significant differences between the trial groups, one-way analysis of variance (ANOVA) was used. After that, the multiple range test designed by Duncan was used for post hoc assessments. The outcomes are presented as the average plus or minus the standard error (SE), and a *P-value* of less than 0.05 is considered statistically significant.

RESULTS

Growth performance and water quality parameters

Water temperature (28°C), pH (7.6–8.2), dissolved oxygen (6.2– 7.3mg/ L), and total ammonia-nitrogen (TAN) levels (0.04–0.07 mg/L) remained at the ideal levels for the Nile tilapia culture, with no substantial variances ($P \leq 0.05$) observed between experimental groups. However, the Nile tilapia given the feed containing 2.0g of probiotic/kg feed (T4) displayed substantially ($P < 0.05$) enhanced final body weight (FBW), WG, daily weight gain (DG), and SGR, in both males and females, compared with control and other treatments ($P \leq 0.05$). Additionally, the highest feed consumption and the best FCR were also recorded in the T4 (Table 1).

Table 1. Growth performance and feed utilization of *O. niloticus* given feed enriched with various concentrations of probiotic for two months

Treatments	T1	T2	T3	T4
Initial weight	115.50±0.29	116.17±0.44	115.83±0.17	114.50±0.29
Final weight	241.33^{d}±1.01	257.67^{c} ±0.30	265.17^{b} ±0.44	277.33^{a} ±1.30
Weight gain	125.50^{d} ±1.32	141.50^{c} ±1.73	149.33^{b} ±0.33	162.80^{a} 3±1.10
Average Daily Gain	2.09^{d} ±0.02	2.46^{c} ±0.03	2.49^{b} ±0.01	2.71^{a} ±0.02
SGR	1.23^{d} ±0.01	1.33^{c} ±0.02	1.38^{b} ±0.00	1.47^{a} ±0.00
Feed intake	258.46^{b} ±1.39	259.24^{b} ±1.26	262.27^{b} ±2.20	269.69^{a} ±1.24
FCR	2.06^{d} ±0.01	1.83^{c} ±0.03	1.76^{b} ±0.01	1.66^{a} ±0.00

Data are expressed as means ± standard error (SE). Values within the same row that possess differing superscript letters indicate a significant difference *(P* < 0.05).

Fish biology

Hepatosomatic index (HSI) and liver health

The hepatosomatic index (HSI), which indicates liver weight relative to total body weight and measures hepatic condition, exhibited no significant variations between the trial groups administered varying probiotic doses. The HSI values recorded for treatments T1, T2, T3, and T4 were 2.03, 2.32, 2.43, and 2.59%, respectively, indicating no adverse effects on liver health due to probiotic supplementation (Fig. 1).

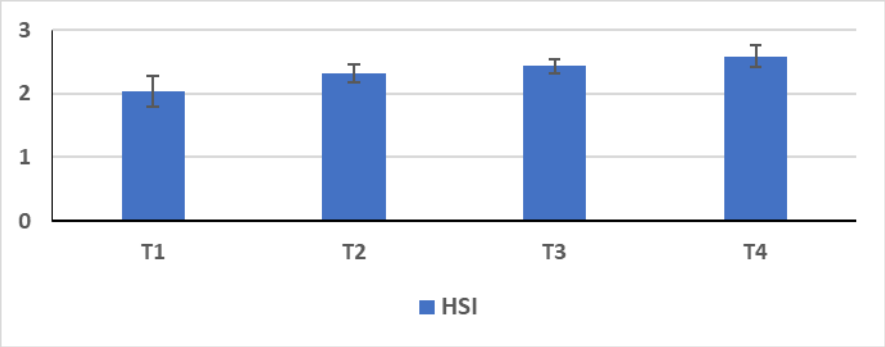

Fig. 1. Hepatosomatic indices of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months

Erythrocyte count, hemoglobin, and hematocrit

Marked variations ($P \leq 0.05$) were seen in red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (Hct, %) values between the control group and *O. niloticus* receiving diets with differing probiotic concentrations (Fig. 2). Fish receiving 1.0 g probiotic/kg diet (T3) exhibited the highest RBC and Hb values compared to other treatments ($P \leq 0.05$). The greatest hematocrit (Hct) concentration was detected in *O. niloticus* given the 2.0g probiotic/kg diet (T4), while the lowest significant Hb and Hctvalues were found in *O. niloticus* receiving 0.5g probiotic/kg diet (T2) ($P \leq 0.05$). Overall, RBC counts showed an increasing trend corresponding with higher probiotic inclusion levels relative to the control group.

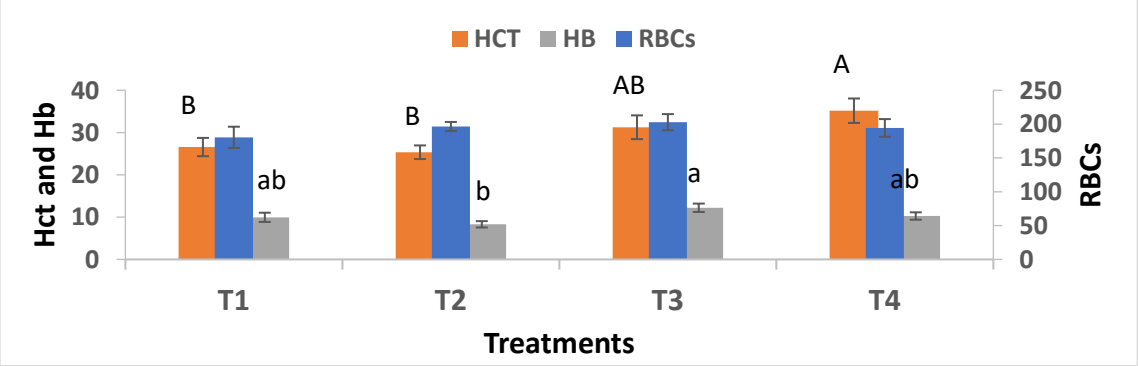

Fig. 2. RBCs (x106/µl), Ht (%), and Hb (g/dl) of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months

Erythrocyte indices

There were notable variations in the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), between the control fish and those that were given different quantities of probiotics in their meals ($P \leq 0.05$) (Fig. 3). Specifically, tilapia fed 2.0g probiotic/kg diet (T4) exhibited a significantly higher MCV in comparison to other trial groups ($P \leq 0.05$). At the same time, alterations in MCH and MCHC, were not statistically significant. These parameters were evaluated over a 60-day feeding period.

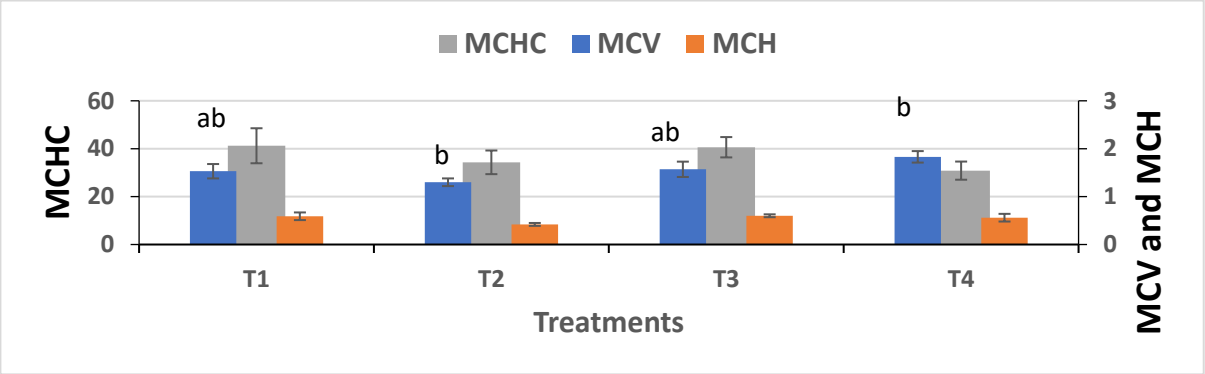

Fig. 3. MCV (µm³), MCH (pg), and MCHC (g/dl) of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months

Blood biochemical parameters

Substantial variances (≤ 0.05) were detected in blood biochemical indices between control fish and those fed diets containing varying probiotic levels (Figs. 4, 5). While probiotic supplementation had no significant effect on globulin and plasma urea concentrations, total plasma protein, and albumin levels were substantially impacted by the addition of nutritional probiotics ($P \leq 0.05$). Additionally, fish fed the 2.0g probiotic/kg diet (T4) exhibited elevated values of AST and ALT in comparison with other treatment groups. The maximum total lipid concentration was noted in *O. niloticus* given feed enriched with the 0.5g probiotic/kg diet (T2), while this variance was not substantial ($P > 0.05$).

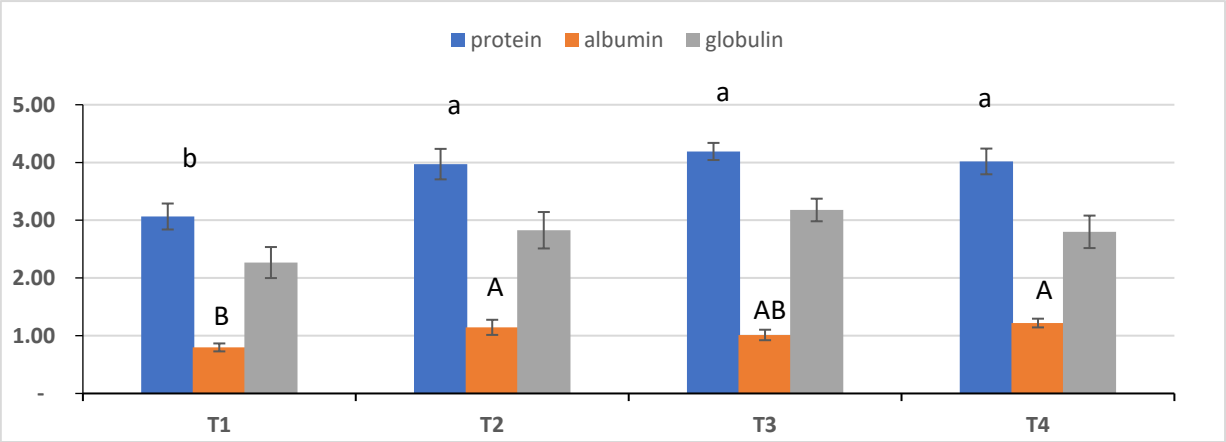

Fig. 4. Protein, albumin, and globulin of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months

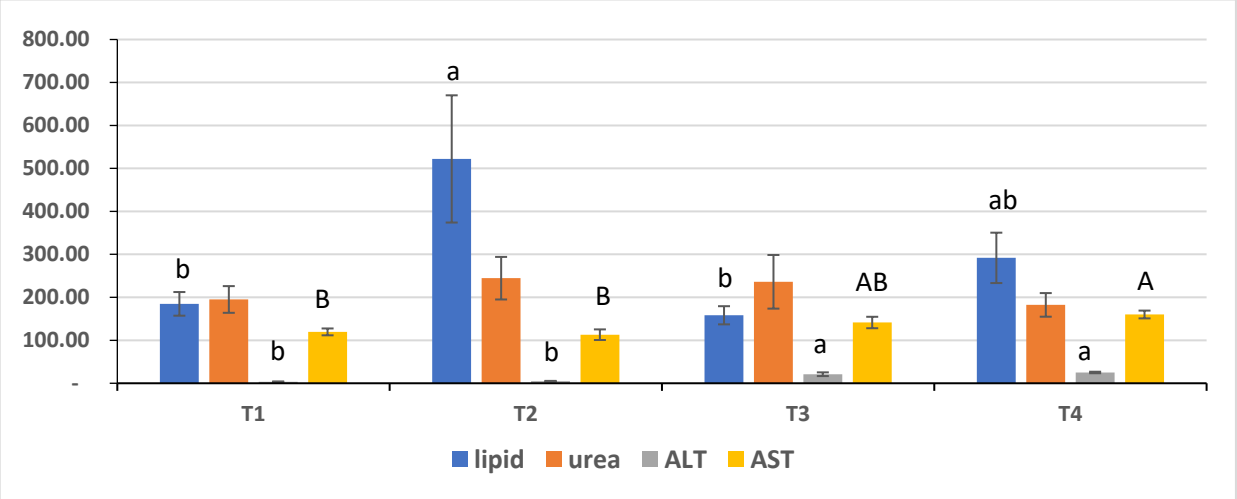

Fig. 5. Lipid, urea, ALT and AST of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months

Histology

Liver

Probiotic supplementation did not induce any notable alterations in liver histology, with hepatocyte structure and nuclear morphology appearing normal and indicative of healthy liver tissue (Fig. 6).

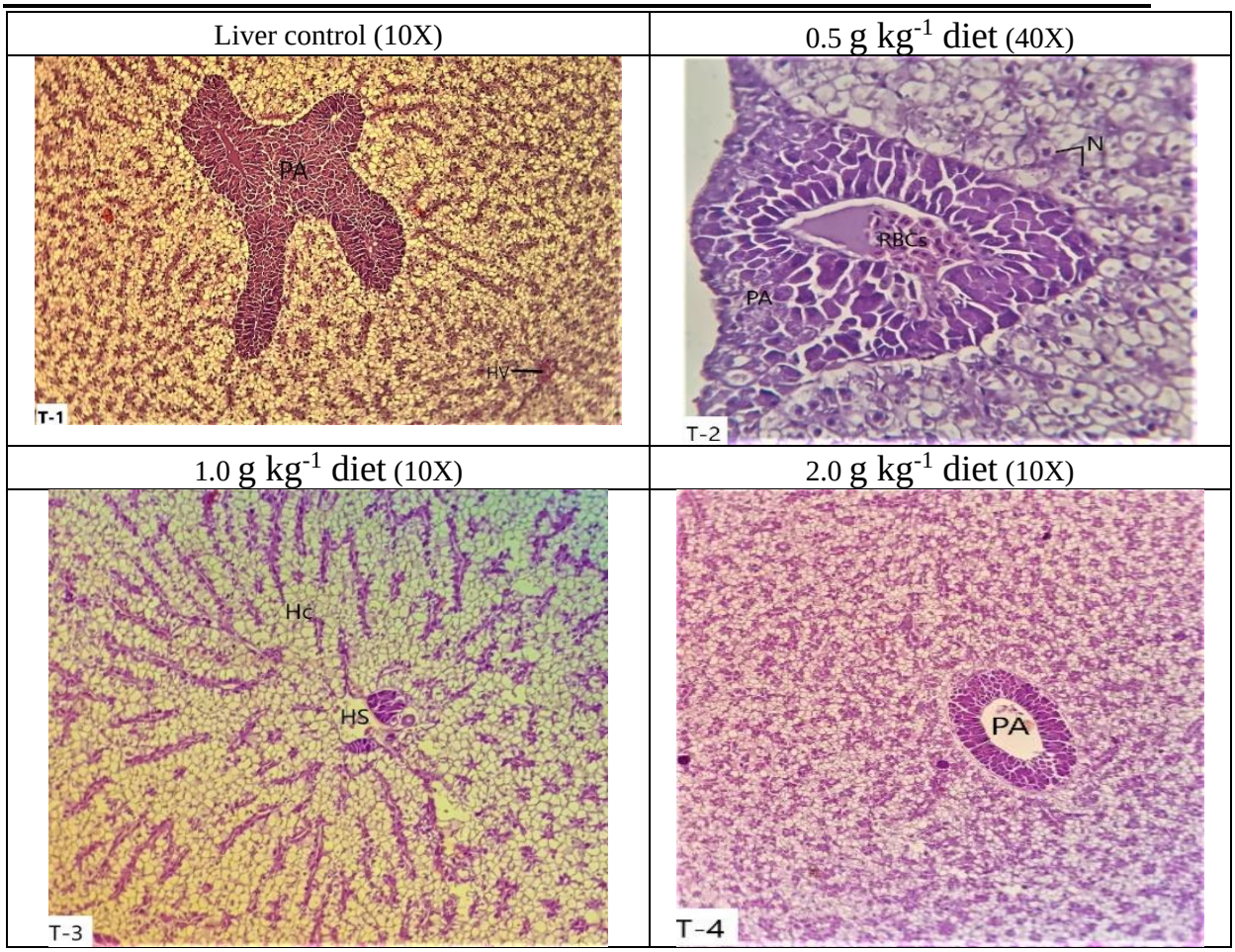

Fig. 6. Livers of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months. HS - Hepatic sinusoid, Hc – Hepatocyte, HV - Hepatic vein, PA Hepatic pancreatic acini, RBCs – red blood cell, N – nuclei, hepatocytes (Hc), hepatic sinusoid (HS), hepatopancreatic acini (HA), nucleus (N)

Intestines

The intestinal tissue exhibited visible changes with probiotic treatment, including an increased number of goblet cells and an expanded surface area, attributed to elongation of the intestinal villi (Fig. 7).

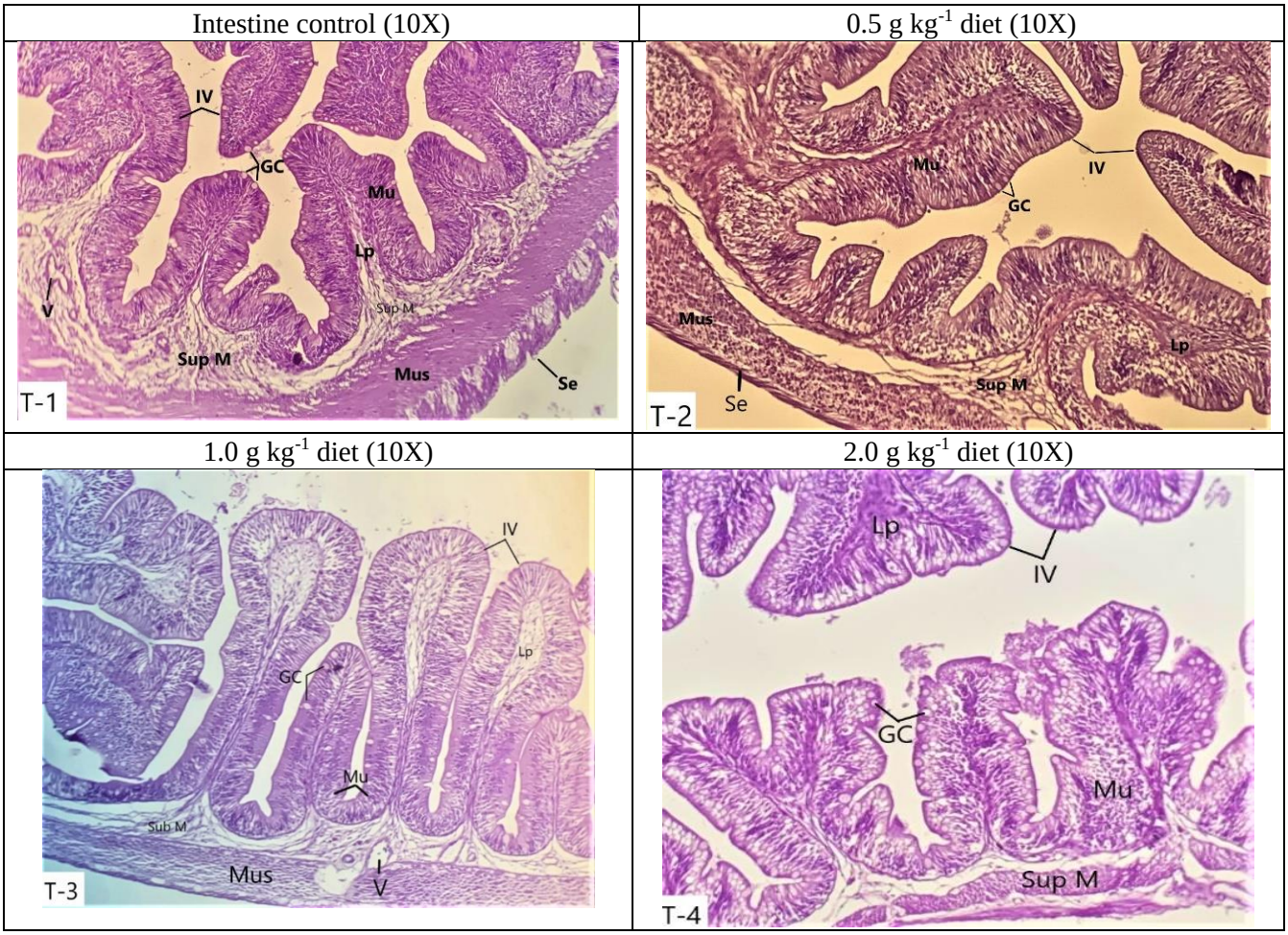

Fig. 7. Intestines of *O. niloticus* were given feed enriched with various concentrations of probiotics for two months. Mus muscular, GC goblet cell, V vein, Mu meucas, Sub M - sup mucaus mucosa (Mu), muscularis (Mus), serosa (Se). The intestinal villi (IV) and Lamina propria (Lp)

DISCUSSION

In the present study, water quality parameters remained unaffected by dietary supplementation with probiotics, suggesting that *B. subtilis* did not negatively influence environmental conditions within the aquaria. This finding aligns with **Billard's (1995)** emphasis that *Bacillus* spp. can enhance water quality by promoting the biodegradation of nitrogenous waste and facilitating mineralization. While water quality itself was stable, its interaction with probiotic efficacy in aquaculture systems has been noted in earlier research **(Jahangiri *et al.*, 2018)**, indicating that favorable environmental conditions may further support the beneficial actions of probiotics.

The observed enhancement in all growth performance parameters in response to probiotic supplementation confirms the positive influence of *B. subtilis* on the Nile tilapia

growth. These findings corroborate those of earlier research showing that tilapia given diets supplemented with *B. subtilis* also showed improvement **(Hassaan *et al.,* 2019)**. The enhancement of aquaculture productivity through probiotics has been attributed to enhanced feed conversion, nutrient digestibility, and modulation of gut microbiota, which collectively optimize energy utilization and promote better body composition and hepatic function **(El-Saadony *et al.,* 2021; Amenyogbe, 2023)**. The current findings also support earlier reports that both dietary and environmental probiotic applications can substantially boost DW, feed consumption, feed conversion efficiency, biomass yield, and survival **(Van Doan *et al.,* 2020; Deng *et al.,* 2022)**.

In harmony with **Hersi *et al.* (2023)**, the growth-promoting effects detected in the current study reinforce the value of probiotics in *O. niloticus* culture. Moreover, the hepatosomatic index (HSI), a physiological marker of lipid metabolism and energy storage, showed no significant variation across the different treatments. This corresponds with the conclusions of **Rahmi *et al.* (2023)**, suggesting that probiotic supplementation at the tested levels does not compromise liver function. The maintenance of stable HSI values further indicates efficient lipid regulation and digestive health **(Pires *et al.,* 2022)**, reinforcing the metabolic stability of fish receiving Bacillus-based diets

Hematological variables are considered vital markers of fish wellbeing, particularly in assessing oxygen-carrying capacity and immune status. Although not statistically significant, a trend toward elevated RBC numbers and Hb concentrations was noted in probiotic-supplemented groups. Similar moderate improvements in hematological profiles have been reported in tilapia given feeds enriched with *Bacillus strains* **(Moustafa *et al.,* 2021; Tachibana *et al.,* 2021)**. The ability of probiotics to modulate blood parameters has also been shown in fish supplemented with *Bacillus cereus* and *Aspergillus oryzae* **(Liu *et al.,* 2017; Dawood *et al.,* 2019)**, reflecting a positive physiological response to probiotic inclusion. These hematological changes are commonly interpreted as adaptive responses to improved dietary quality and overall immune stimulation **(Kutlu *et al.,* 2020; Ghosal *et al.,* 2021)**.

Biochemical analyses revealed a substantial improvement in total protein and albumin levels with increasing probiotic inclusion, indicating improved protein metabolism and immunocompetence. Comparable outcomes have been reported in various fish species, for instance *Cyprinus carpio* **(Gupta *et al.,* 2016)** and *Dicentrarchus labrax* **(Schaeck *et al.,* 2017)**. Earlier studies have recorded relative output such as that of **Wang *et al.* (2008)** and **Sutthi *et al.* (2018)**. Moreover, the probiotic-induced improvements in hepatic condition observed in the current research follow the results of **Hossain *et al.* (2022)**, who noted that probiotics can mitigate liver lipid accumulation and thereby reduce the risk of metabolic disorders.

Histological evaluation of the anterior intestine revealed notable improvements in gut structure, characterized by increased villus length and higher goblet cell counts in fish fed probiotic diets. These modifications indicate an expanded absorptive surface and stronger mucosal defense, contributing to improved nutrient uptake and barrier integrity. These results align with other study demonstrating the beneficial influence of probiotics on the intestinal morphology of tilapia (**Obianwuna *et al.*, 2023)**. Goblet cell proliferation, stimulated by probiotic administration, enhances mucus secretion and bolsters mucosal immunity, offering protection against enteric pathogens **(Duangnumsawang *et al.*, 2021; Gyawali *et al.*, 2023)**. Our results also corroborate earlier studies that reported elevated goblet cell activity and improved gut histoarchitecture in response to probiotic feeding **(Jayaprakash & Parvathi, 2019; Oliveira *et al.*, 2022)**, further emphasizing the function of probiotics in maintaining intestinal health and functional integrity **(Al-Yassir *et al.*, 2021)**.

CONCLUSION

The current investigation demonstrates that the nutritional addition of *B. subtilis* in *O. niloticus* feed significantly enhances growth variables, feed utilization, biochemical parameters, and intestinal morphology of *O. niloticus*, without negatively affecting water quality or the hepatosomatic index. The insertion of *B. subtilis* at 2.0g /kg yielded the most favorable outcomes in terms of growth rate, protein utilization, and intestinal health, as evidenced by increased villus height and goblet cell density. Furthermore, improvements in total protein and albumin levels suggest a positive influence on systemic metabolism and immune function. The existing conclusions suggest supplementing *B. subtilis* as a functional probiotic additive in tilapia aquaculture and recommend a supplementation level of 2.0g/ kg for optimal physiological and productive benefits.

REFERENCES

Al-Yassir, F.; Khoder, G.; Sugathan, S.; Saseedharan, P.; Al Menhali, A. and Karam, S. M. (2021). Modulation of Stem Cell Progeny by Probiotics during Regeneration of Gastric Mucosal Erosions. *Biology*, *10*(7), 596.

Amenyogbe, E. (2023). Application of probiotics for sustainable and environment-friendly aquaculture management - A review. *Cogent Food & Agriculture*, *9*(1), 2226425.

Ayisi, C. L.; Apraku, A. and Afriyie, G. (2017). A Review of Probiotics, Prebiotics, and Synbiotics in Crab: Present Research, Problems, and Future Perspective. *Journal of Shellfish Research*(3), 799-806, 798.

Britton, J. L. (1963). The word problem. Annals of Mathematics, 77(1), 16-32.

Busher, J.T. (1990). Albumin and Globulin. In Clinical Methods: The History, Physical and Laboratory Examinations.

Carlos, M. H. (1988). Growth and survival of bighead carp (*Aristichthys nobilis*) fry fed at different intake levels and feeding frequencies. *Aquaculture*, 68(3), 267–276.

Dacie, J.V. and Lewis, S.M. (1991). Practical Haematology. Pub.: ChurchhillL Livingstone. London. pp1-73.

Dawood, M. A. O.; Eweedah, N. M.; Moustafa Moustafa, E. and Shahin, M. G. (2019). Effects of feeding regimen of dietary *Aspergillus oryzae* on the growth performance, intestinal morphometry and blood profile of Nile tilapia (*Oreochromis niloticus*). *25*(5), 1063-1072.

Deng, Y.; Verdegem, M. C. J.; Eding, E. and Kokou, F. (2022). Effect of rearing systems and dietary probiotic supplementation on the growth and gut microbiota of Nile tilapia (*Oreochromis niloticus*) larvae. *Aquaculture, 546*, 737297.

El-Saadony, M. T.; Alagawany, M.; Patra, A. K.; Kar, I.; Tiwari, R.; Dawood, M. A. O. and Abdel-Latif, H. M. R. (2021). The functionality of probiotics in aquaculture: An overview. *Fish and Shellfish Immunology, 117*, 36-52.

FAO. (2024). World Fisheries and Aquaculture. *Blue Transformation in action*.

Ghosal, I.; Mukherjee, D. and Chakraborty, S. B. (2021). The effects of four plant extracts on growth, sex reversal, immunological and haemato-biochemical parameters in Nile tilapia, *Oreochmomis niloticus* (Linnaeus, 1758). *Aquaculture Research, 52*(2), 559-576.

Ghosh, K.; Harikrishnan, R.; Mukhopadhyay, A. and Ringø, E. (2023). Fungi and Actinobacteria: Alternative Probiotics for Sustainable Aquaculture. *Fishes, 8*(12), 575.

Gupta, A.; Gupta, P. and Dhawan, A. (2016). *Cyprinus carpio*as a water additive improved immune response of *Aeromonas hydrophila* and disease resistance against *Aeromonas hydrophila*. *Aquaculture Reports, 4*, 86-92.

Gyawali, I.; Zhou, G.; Xu, G.; Li, G.; Wang, Y.; Zeng, Y. and Jiang, Q. (2023). Supplementation of microencapsulated probiotics modulates gut health and intestinal microbiota. *Food Science and Nutrition, 11*(8), 4547-4561.

Hassaan, M. S.; El-Sayed, A. I. M.; Soltan, M. A.; Iraqi, M. M.; Goda, A. M.; Davies, S. J. and Ramadan, H. A. (2019). Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, *Oreochromis niloticus*. *Aquaculture, 503*, 282-292.

Henry R.J. (1974). Clinical chemistry, Principle and Techniques, 2nd edition, Harper and Row, New York, 822 p.

Hersi, M. A.; Genc, E.; Pipilos, A. and Keskin, E. (2023). Effects of dietary synbiotics and biofloc meal on the growth, tissue histomorphology, whole-body composition and intestinal microbiota profile of Nile tilapia (*Oreochromis niloticus*) cultured at different salinities. *Aquaculture, 570*, 739391.

Hossain, M. A.; Sutradhar, L.; Sarker, T. R.; Saha, S. and Iqbal, M. M. (2022). Toxic effects of chlorpyrifos on the growth, hematology, and different organs

histopathology of Nile tilapia, *Oreochromis niloticus*. *Saudi Journal of Biological Sciences, 29*(7), 103316.

Jahangiri, L. and Esteban, M. Á. (2018). Administration of Probiotics in the Water in Finfish Aquaculture Systems: A Review. *Fishes, 3*(3), 33.

Kutlu, H.; Avci, E. and Özyurt, F. (2020). White blood cells detection and classification based on regional convolutional neural networks. *Medical Hypotheses, 135*, 109472.

Liu, H.; Wang, S.; Cai, Y.; Guo, X.; Cao, Z.; Zhang, Y. and Zhou, Y. (2017). Dietary administration of *Bacillus subtilis* HAINUP40 enhances growth, digestive enzyme activities, innate immune responses and disease resistance of tilapia, *Oreochromis niloticus*. *Fish and Shellfish Immunology, 60*, 326-333.

Mahi, T. F.; Chowdhury, G.; Hossain, M. A.; Baishnab, A. K.; Schneider, P. and Iqbal, M. M. (2022). Assessment of Lead (Pb) Toxicity in Juvenile Nile Tilapia, *Oreochromis niloticus*—Growth, Behaviour, Erythrocytes Abnormalities, and Histological Alterations in Vital Organs. *Toxics, 10*(12), 793.

Moustafa, E. M.; Farrag, F. A.; Dawood, M. A. O.; Shahin, K.; Hamza, A.; Decamp, O. and Omar, A. A. (2021). Efficacy of Bacillus probiotic mixture on the immunological responses and histopathological changes of Nile tilapia (*Oreochromis niloticus, L*) challenged with *Streptococcus iniae*. *Aquaculture Research, 52*(5), 2205-2219.

Munkittrick, K. R. and Dixon, D. G. (1988). Growth, fecundity, and energy stores of white sucker (Catostomus commersoni) from lakes containing elevated levels of copper and zinc. *Canadian Journal of Fisheries and Aquatic Sciences, 45*(8), 1355-1365.

Naiel, M. A. E.; Farag, M. R.; Gewida, A. G. A.; Elnakeeb, M. A.; Amer, M. S. and Alagawany, M. (2021). Using lactic acid bacteria as an immunostimulants in cultured shrimp with special reference to *Lactobacillus spp. Aquaculture International, 29*(1), 219-231.

Naiel, M. A. E.; Shehata, A. M.; El-Kholy, A. I.; El-Naggar, K.; Farag, M. R. and Alagawany, M. (2022). The mitigating role of probiotics against the adverse effects of suboptimal temperature in farmed fish: A review. *Aquaculture, 550*, 737877.

Obianwuna, U. E.; Agbai Kalu, N.; Wang, J.; Zhang, H.; Qi, G.; Qiu, K. and Wu, S. (2023). Recent Trends on Mitigative Effect of Probiotics on Oxidative-Stress-Induced Gut Dysfunction in Broilers under Necrotic Enteritis Challenge: A Review. *Antioxidants, 12*(4), 911.

Oliveira, F. C.; Dichoff, K. R. Y.; Eurico, F. C.; Wesley, S. d. S. and and de Campos, C. M. (2022). Probiotic, prebiotic and synbiotics supplementation on growth performance and intestinal histomorphometry *Pseudoplatystoma reticulatum* larvae. *Journal of Applied Aquaculture, 34*(2), 279-293.

Pires, G. K. G.; Xavier, D. T. O.; Souza, H. B. d.; Castro, V. S. d.; Rossetto, J. F.; Bittencourt, F. and Signor, A. (2022). Probiotic in diets for Nile tilapia (*Oreochromis niloticus*) fingerlings. *Research, Society and Development, 11*(9), e6211930987.

Rahmi, R.; Akmal, A.; Nisaa, K.; Sudrajat, I.; Relatami, A. N. R.; Tampangallo, B. R. and Ikbal, M. (2023). Tilapia growth performance and body physical index given synbiotic feed at different salinities. *IOP Conference Series: Earth and Environmental Science, 1137*(1), 012029.

Sarker, B.; Das, B.; Chakraborty, S.; Hossain, M. A.; Alam, M. M. M.; Mian, S. and Iqbal, M. M. (2022). Optimization of 17α-methyltestosterone dose to produce quality mono-sex Nile tilapia *Oreochromis niloticus*. *Heliyon, 8*(12).

Schaeck, M.; Reyes-López, F. E.; Vallejos-Vidal, E.; Van Cleemput, J.; Duchateau, L.; Van den Broeck, W. and Decostere, A. (2017). Cellular and transcriptomic response to treatment with the probiotic candidate Vibrio lentus in gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. *Fish and Shellfish Immunology, 63*, 147-156.

Schmit, J.M. (1964). Colorimetric determination of total lipids with sulpho-sphovanillic Mixture. Ph.D. *Thesis*, Lyon:39 p.

Sumon, M. A. A.; Molla, M. H. R.; Hakeem, I. J.; Ahammad, F.; Amran, R. H.; Jamal, M. T. and Hasan, M. T. (2022). Epigenetics and Probiotics Application toward the Modulation of Fish Reproductive Performance. *Fshes, 7*(4), 189.

Sutthi, N.; Thaimuangphol, W.; Rodmongkoldee, M.; Leelapatra, W. and Panase, P. (2018). Growth performances, survival rate, and biochemical parameters of Nile tilapia (*Oreochromis niloticus*) reared in water treated with probiotic. *Comparative Clinical Pathology, 27*(3), 597-603.

Tachibana, L.; Telli, G. S.; Dias, D. d. C.; Gonçalves, G. S.; Guimarães, M. C.; Ishikawa, C. M. and Ranzani-Paiva, M. J. T. (2021). *Bacillus subtilis and Bacillus licheniformis* in diets for Nile tilapia (*Oreochromis niloticus*): Effects on growth performance, gut microbiota modulation and innate immunology. *52*(4), 1630-1642.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor Ann. Clin. Biochem., 6: 24-25.

Van Doan, H. ; **Hoseinifar, S. H.** ; **Ringø, E.** ; **Ángeles Esteban, M.** ; **Dadar, M.; Dawood, M. A. and Faggio, C. (2020).** Host-associated probiotics: a key factor in sustainable aquaculture. Reviews in fisheries science & aquaculture, 28(1), 16-42.

Walakira, J. K.; Hinrichsen, E.; Tarus, V.; Langi, S.; Ibrahim, N. A.; Badmus, O. and Baumüller, H. (2023). Scaling aquaculture for food security and employment in Africa–Insights from Egypt, Kenya and Nigeria. *ZeF Center for Development Research, University of Bonn*.

Wang, Y.-B.; Tian, Z.-Q.; Yao, J.-T. and Li, W.-f. (2008). Effect of probiotics, Enteroccus faecium, on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Aquaculture, 277*(3), 203-207.

Yilmaz, S.; Yilmaz, E.; Dawood, M. A. O.; Ringø, E.; Ahmadifar, E. and Abdel-Latif, H. M. R. (2022). Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review. *Aquaculture, 547*, 737514.