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IMPACT OF *BACILLUS TROPICUS* PROBIOTIC ON WATER QUALITY, HAEMATOLOGICAL INDICES AND LIVER ENZYME FUNCTIONS IN *OREOCHROMIS NILOTICUS.*

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ABSTRACT

This study explores the impact of *Bacillus tropicus*, a probiotic strain isolated from fermented rice on water quality, haematological parameters and liver enzyme activities in *Oreochromis niloticus* over varying experimental durations. Fish treated with the probiotic exhibited notably lower ammonia and nitrite concentrations in tank water compared to the control. Significant (P<0.05) improvements in red blood cells and white blood cell counts, as well as haemoglobin levels were observed in probiotic-fed fish. Furthermore, a marked reduction in AST and ALT enzyme level was noted in probiotic fed fish group over the control suggesting enhanced liver health in the experimental group.

INTRODUCTION

Aquaculture has become one of the fastest expanding food-producing sectors globally with countries like China, India, Indonesia, Thailand and the Philippines contributing approximately 80% to global production (FAO, 1995). Recent data from FAO (2024) indicates that worldwide aquaculture and fisheries production reached an all-time high of 223.2 million tonnes in 2022, reflecting a 4.4% increase since 2020. India stands as the third-largest fish producer and the second-largest aquaculture producer after China. Nile tilapia (*Oreochromis niloticus*) is an ideal species for aquaculture due to its rapid growth, adaptability to diverse water conditions, and disease resistance, making it favourable for small-scale farming operations.

Probiotics is live microorganisms that confer health benefits and are extensively used in various fields including aquaculture, for promoting immunity, enhancing growth, and improving water quality (Gomez-Gil *et al.*, 2000). They help to maintain microbial balance by reducing pathogenic bacteria and are considered environmentally friendly alternatives to antibiotics (Jahangiri and Esteban, 2018). In fish, probiotics contribute to improved digestion through enzyme secretion, enhanced nutrient absorption (Sahu *et al.*, 2008) and nitrogen waste management through the processes such as nitrification and de-nitrification (Rout *et al.*, 2017; Liu *et al.*, 2020). Incorporating probiotics into fish diets can also reduce feed costs while boosting growth rates, as observed in African catfish fed *Lactobacillus acidophilus* (Al-Dohail *et al.*, 2009). Intestinal microbiota play an essential role in producing biologically active substances like enzymes, amino acids, and vitamins that support overall fish health (Wang, 2007). Probiotics also stimulate lymphocyte production and enhance antibody responses (Al-Dohail *et al.*, 2009). Hematological indicators such as RBC, WBC, and haemoglobin levels are commonly used to evaluate fish health and nutrition (Ologhobo, 1992). Elevated levels of liver enzymes like ALT and AST often signal liver dysfunction or cellular damage (Kumar *et al.*, 2011), and their measurement is vital for assessing physiological stress in fish. Hence, the present study aims to find out the impact of isolated probiotic bacteria *Bacillus tropicus* from fermented rice on water quality, haematological parameters and liver enzyme activities on *O.niloticus*.

1. MATERIALS AND METHODS

Oreochromis niloticus was the experimental animal belongs to the family Cichlidae and an enhanced strain of Nile Tilapia. *Oreochromis niloticus* fingerlings with the average weight of 4 ± 0.1gm and length of 5 ± 0.1cm were purchased from Multi Species Aquaculture Complex of MPEDA- RGCA, Vallarpadam, Kochi, Ernakulum district, Kerala, India. The experimental fish were transported to the experimental tank in double polyethylene bags filled with oxygenated water. The fish were acclimatized for a month in a tank and fed with commercial fish feed. *Bacillus tropicus* is a gram positive, spore- forming facultative anaerobic *Bacillus* species, isolated from fermented rice. *Bacillus tropicus* produces antimicrobial metabolites which inhibit the growth of fish and human pathogens and other bioactive compounds.

2.1 EXPERIMENTAL DESIGN

The two biofloc tanks such as Control Tank and Experimental Tank were selected for the present study. The fish in the control tank was fed only with commercial feed without probiotics and fish in the Experimental tank was fed with commercial fish feed along with 0.5mg of isolated probiotic *Bacillus tropicus*. The feed was given early in the morning (6 am to 7 am) and evening (5 pm to 6pm) regularly at the rate of 3% body weight of fish per day. The experiment was carried out for the period of 120 days and the samples were collected from the control tank and the experimental tank on 30th, 60th 90th and 120th day of the experimental period for further analysis.

The water parameters were estimated by the methods suggested by Golterman and Clymo (1969); Wetzel and Likens (1979) and APHA (1998). Haematological parameters such as Red Blood cells (RBC), White Blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) were recorded in freshwater fish *O. niloticus* from the control tank and the experimental tank. Blood samples were collected after 24 hours starvation period from caudal vein using 2ml syringes and gauge hypodermic needles. Blood samples were collected randomly from the control tank and the experimental tank on 30th, 60th, 90th and 120th day of the experimental period. The collected blood was transferred into eppendrof tube of 2ml capacity and stored in refrigerator for further analysis (Anderson and Klontz, 1965). RBC and WBC count was done with Neubauer chamber (Davidson and Henry, 1974). The Haemoglobin content was determined by using Haemoglobinometer suggested by the method of Blaxhall and Daisley, (1973). AST and ALT are biological catalyst. The assay of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity are determined based on the method suggested by Reitman and Frankel, 1957; Huang, *et al.*, 2006) were recorded on 30th day, 60th day, 90th day and 120th day of the experimental period and data were analyzed Using One way ANOVA.

2. RESULTS

Bacillus tropicus bacteria were isolated from fermented rice and fed with the *O. niloticus* fish for different experimental periods and water quality parameters such as ammonia and nitrite levels, haematological parameters like RBC, WBC, haemoglobin and liver enzyme such as AST and ALT activities in *O. niloticus* were analyzed on 30th day, 60th day, 90th day and 120th day of the experimental periods and results were tabulated. The ammonia level was significantly increased from 0.53 ± 0.02^a mg/l (30th day) to 0.66 ± 0.01^c mg/l (120th day) in the control tank whereas, ammonia level in the experimental tank was significantly decreased from 0.5 ± 0.01^a mg/l (30th day) to 0.45 ± 0.01^b mg/l (120th day). Nitrite level in the control tank was significantly increased from 0.26 ± 0.02^a mg/l (30th day) to 0.37 ± 0.01^b mg/l (120th day) whereas, the nitrite level in the experimental tank was gradually decreased from 0.29 ± 0.01^a mg/l (30th day) to 0.26 ± 0.01^b mg/l (120th day) (Table-1)

Table 1: Changes in the Ammonia and Nitrite level of *Oreochromis niloticus* rearing tank water treated with probiotics *B. tropicus* after different experimental period.

EXPERIMETAL PERIOD (DAYS)	Ammonia (mg/l)		Nitrite(mg/l)	
	CONTROL TANK	EXPT. TANK	CONTROL TANK	EXPT. TANK
30	0.53 ± 0.02^a	0.5 ± 0.01^a	0.26 ± 0.02^a	0.29 ± 0.1^a
60	0.58 ± 0.02^b	0.47 ± 0.02^{ab}	0.34 ± 0.04^b	0.27 ± 0.02^a
90	0.6 ± 0.01^b	0.46 ± 0.04^{ab}	0.36 ± 0.03^b	0.25 ± 0.04^a
120	0.66 ± 0.01^c	0.45 ± 0.01^b	0.37 ± 0.01^b	0.26 ± 0.02^a

Each value is mean ± SD; n=3; Mean values within the same row sharing the different alphabetical superscripts are statistically significant level at $P< 0.05$ (One way ANOVA and subsequent *post hoc* multiple comparison with DMRT).

The Red blood corpuscle (RBC) count was significantly increased from $2.11\times10^6 \pm 0.01^a$ mm^3 (30 day) to 6. The WBC count was also increased from $2.11\times10^6 \pm 0.01^a$ mm^3 $6.6 \times 10^6 \pm 0.3^d$ mm^3 (30 day) in the control tank, whereas the RBC count was significantly increased from $4.4\times10^6 \pm 0.2^a$ mm^3 (30th day) to $8.4\times10^6 \pm 0.4^c$ mm^{3}on 120th day of the experimental tank. $2.23\times10^5 \pm 0.02^a$ mm^3 (30th day) to $5.83\times10^5 \pm 0.03^c$ mm^3 (120th day) in control group and WBC count in the experimental group was also significantly increased from $4.4\times10^5 \pm 0.2^a$ mm^3 (30th day) to $7.93\times10^5 \pm 0.03^c$ mm^3 (120th day) (Table-2).

Table 2: Changes in the RBC and WBC count of *Oreochromis niloticus* fed with isolated probiotic bacteria *B. tropicus* after different experimental period.

EXPERIMETAL PERIOD (DAYS)	RBC Count (mm^3)		WBC Count (mm^3)	
	CONTROL TANK	EXPT. TANK	CONTROL TANK	EXPT. TANK
30	$2.11\times10^6 \pm 0.01^a$	$4.4\times10^6 \pm 0.2^a$	$2.23\times10^5 \pm 0.02^a$	$4.4\times10^5 \pm 0.2^a$
60	$4.13\times10^6 \pm 0.03^b$	$6.7\times10^6 \pm 0.3^b$	$4\times10^5 \pm 2^{ab}$	$5.7\times10^5 \pm 0.1^b$

90	$5.4\times10^{6} \pm 0.2^{c}$	$8.1\times10^{6} \pm 0.1^{c}$	$6.03\times10^{5} \pm 0.02^{bc}$	$7.7\times10^{5} \pm 0.2^{c}$
120	$6.6\times10^{6} \pm 0.3^{d}$	$8.4\times10^{6} \pm 0.4^{c}$	$5.83\times10^{5} \pm 0.03^{c}$	$7.93\times10^{5} \pm 0.03^{c}$

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts are statistically significant level at P< 0.05 (one way ANOVA and subsequent *post hoc* multiple comparison with DMRT)

The haemoglobin level in the control group was observed as 2.3 ± 0.2^{a} g/ dL as on 30th day and 70.13 ± 0.01^{d} g/ dL on 120th day of the experimental period. Whereas the haemoglobin level in the experimental tank was significantly increased from 75.42 ± 0.01^{a} g/ dL (30th day) to 89.6 ± 0.2^{d} g/ dL on 120th day of experimental period (Table-3)

Table 3: Changes in the haemoglobin level of *Oreochromis niloticus* fed with isolated probiotic bacteria *B.tropicus* after different experimental period.

EXPERIMETAL PERIOD (DAYS)	Haemoglobin Level (g/ dL)	
	CONTROL TANK	EXPT. TANK
30	2.3 ± 0.2^{a}	3 ± 0.1^{a}
60	2.9 ± 0.1^{b}	3.8 ± 0.01^{b}
90	3.2 ± 0.2^{c}	4.1 ± 0.1^{c}
120	3.9 ± 0.01^{d}	5.9 ± 0.1^{d}

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts are statistically significant level at P< 0.05 (one way ANOVA and subsequent *post hoc* multiple comparison with DMRT).

The AST and ALT activities in the control and Experimental groups of *O.niloticus* were analyzed and presented in the Table.4. AST activity in the control group was increased from 58.58 ± 0.05^{a} µg/ml (30th day) to 70.13 ± 0.01^{d} µg/ml (120th day) whereas, AST activity in the experimental group was significantly decreased from 75.42 ± 0.01^{a} µg/ml (30th day) to 69.6 ± 0.2^{d} µg/ml (120th day) .The ALT activity was increased from 42.83 ± 0.02^{a} µg/ml (30th day) and 51.77 ± 0.02^{d} µg/ml (120th day) in the control group, whereas, the ALT activity in the experimental group was significantly decreased from 64.13 ± 0.03^{a} µg/ml (30th day) to 50.27 ± 0.02^{d} µg/ml on 120th day of the experimental period.

Table 4: Changes in the AST and ALT levels of *Oreochromis niloticus* fed with isolated probiotic bacteria *B.tropicus* after different experimental period.

EXPERIMETAL PERIOD (DAYS)	AST level (µg/ml)		ALT level (µg/ml)	
	CONTROL TANK	EXPT. TANK	CONTROL TANK	EXPT.TANK
30	58.58 ± 0.05^{a}	75.42 ± 0.01^{a}	42.83 ± 0.02^{a}	64.13 ± 0.03^{a}
60	66.47 ± 0.02^{b}	68.58 ± 0.02^{b}	51.09 ± 0.01^{b}	65.43 ± 0.01^{b}
90	70.43 ± 0.03^{c}	61.79 ± 0.02^{c}	54.57 ± 0.03^{c}	55.97 ± 0.01^{c}
120	70.13 ± 0.01^{d}	69.6 ± 0.2^{d}	51.77 ± 0.02^{d}	50.27 ± 0.02^{d}

Each value is mean ± SD; *n*=3; Mean values within the same row sharing the different alphabetical superscripts are statistically significant level at P< 0.05 (one way ANOVA and subsequent *post hoc* multiple comparison with DMRT)

DISCUSSION

Water quality is a fundamental factor influencing the success of aquaculture operations. According to Negm *et al*. (2021), maintaining optimal water parameters is essential for sustaining healthy fish populations. Incorporating probiotics into aquaculture systems has been widely shown to enhance water conditions and promote better growth in *Oreochromis* species (El-Kady *et al*., 2022). One of the major challenges in aquaculture is the accumulation of metabolic wastes such as uneaten feed and faeces, which contribute to the production of toxic compounds like ammonia. Elevated ammonia concentrations are harmful, as they can damage fish gills, impair oxygen exchange, disrupt blood pH balance, suppress immune responses and reduce appetite. Nitrite accumulation is equally problematic. It enters the fish bloodstream through the gills and converts haemoglobin into met-haemoglobin a form that cannot transport oxygen leading to tissue hypoxia, lethargy, gill discoloration and long-term immune suppression or reproductive issues. In the current study, the gradual decrease in ammonia from 0.5 ± 0.01 mg/l on day 30 to 0.45 ± 0.01 mg/l on day 120 in the probiotic-treated group indicates that *Bacillus tropicus* was effective in reducing nitrogenous waste, likely by promoting nitrification and organic waste breakdown.

These observations align with findings by Khademzade *et al.* (2020), who demonstrated that *Pediococcus acidilactici* and *Bacillus* species reduced nitrogenous waste in shrimp ponds. The nitrite concentrations in the present study also declined in the probiotic tank, supporting the idea that microbial nitrifiers such as *Nitrosomonas* and *Nitrobacter* played a role in the complete conversion of ammonia to nitrate under probiotic influence. Conversely, the control tank showed a continuous rise in nitrite from 0.26 to 0.37 mg/l over the 120-day period, possibly indicating incomplete nitrification and lack of beneficial microbial activity.

Haematological analysis is a valuable method for assessing fish health and detecting underlying infections (Svobodova *et al.*, 1991). In the current experiment, the administration of *B. tropicus* resulted in significant increases in red blood cell (RBC), white blood cell (WBC), and haemoglobin (Hb) levels. These improvements suggest enhanced physiological status and immune competence in the treated fish. Supporting studies by Aly *et al.* (2008a, 2008b) showed that *B. pumilus* improved haematocrit values and leukocyte profiles in *O. niloticus*. Likewise, Irianto and Austin (2002) demonstrated that probiotic strains from *B. gonionotus* elevated erythrocyte and leukocyte counts, likely aiding in non-specific immune responses via increased neutrophil and macrophage activity. Sampath *et al.* (2008) also emphasized that enhanced blood cell indices are indicative of good fish health. The elevated WBC levels observed may reflect increased immune activation, reducing the risk of disease (Queiroz *et al.,* 1998). Thus, *B. tropicus* appears to contribute positively to the haematological status and disease resilience of *O. niloticus.*

ALT and AST are key liver enzymes involved in amino acid and energy metabolism, serving as indicators of liver integrity. Elevated enzyme levels often point to liver cell damage, oxidative stress, or toxic exposure. Kamgar *et al.* (2013) reported higher ALT and AST activities in control groups compared to probiotic-treated fish, suggesting that untreated fish may suffer from hepatic stress. Similarly, Hussein *et al.* (2000) observed elevated transaminases in fish exposed to aflatoxins, further indicating liver injury. In contrast, our study recorded significantly reduced ALT and AST levels in fish treated with *B. tropicus*, consistent with the results of Jessus *et al.* (2002) and Safinaz (2006). While enzyme activity in the control group increased over time possibly due to accumulating physiological or environmental stress the probiotic-treated group exhibited a steady decline in these markers, indicating better metabolic balance and liver protection.

CONCLUSION

Good water quality improves the efficacy of fish culture. Haematological parameters are useful tool that helps to assess the health status of fish. ALT and AST levels serve as reliable biomarkers of liver function and stress. Ongoing research emphasizes water treatment systems and genetic markers to enhance resistance and maintain sustainable tilapia farming. Probiotic inclusion initially activates the hepatic metabolism reflecting physiological adaptation , overtime it stabilizes the liver enzyme activity , suggesting hepatic protection and reduced stress. Monitoring the water quality, hematological parameters and enzyme activities helps the aquaculturists and researchers to assess fish health and evaluate the effects of nutritional interventions.

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