P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.108

Probiotic Benefits in Nile Tilapia (*Oreochromis niloticus*) Aquaculture: Growth, Immunity and Pathogen Resistance

Pang Sing Tung [1], Nur Nashyiroh Izayati Mastor [1], Gazali Salim [2], Nur Indah Septriani [3] and Julian Ransangan [1,4,*]

[1]Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia
[2]Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Borneo University, Amal Lama No. 1 Tarakan, North Kalimantan, Indonesia
[3]Faculty of Biology, Universitas Gadjah Mada, Jalan Teknika Selatan, Sinduadi, Mlati, Sleman, Yogyakarta, Indonesia 55281
[4]Higher Institution Centre of Excellence (HICoE), Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, Kota Kinabalu, Sabah 88400, Malaysia
***Corresponding author:** liandra@ums.edu.my

Article History: 25-158 Received: 28-Jun-25 Revised: 12-Aug-25 Accepted: 18-Aug-25 Online First: 19-Sep-25

ABSTRACT

The excessive use of antibiotics in aquaculture has improved short-term fish survival but contributed to antibiotic resistance, raising long-term food safety concerns due to potential residue transmission through the food chain. A sustainable solution to combat fish diseases can be achieved by integrating good husbandry practices with prophylactic agents like immuno-stimulants and probiotics. Probiotics, known for their ability to balance intestinal flora, enhance host health, and boost immunity, have long been used in human and animal diets. The present study evaluates the effects of probiotics sourced from freshwater and marine fish intestines on the growth, survival, and immunity of Nile tilapia (*Oreochromis niloticus*) juveniles under bacterial pathogen challenge. The lethal dose (LD_{50}) of *Aeromonas hydrophila* (strain A10) was determined as 8.13 x 10^{11} for the Nile tilapia juveniles. Probiotic candidates, derived from goldfish (*Carassius auratus*) and marine catfish (*Hexanematichthys sagor*), were applied individually and as mixtures on fish feed. A 14-day *in vivo* trial involving 17 treatment groups, including controls and probiotic combinations, was conducted in triplicate. Post-challenge with *A. hydrophila* LD_{50} revealed all fish survived; however, those treated with mixed probiotics (*Shewanella algae*, *Enterobacter cloacae*, and *Bacillus thuringiensis*) exhibited reduced leukocyte counts but higher lymphocyte counts compared to controls and single-probiotic groups. Additionally, fish treated with mixed probiotics showed a lower feed conversion rate (FCR), indicating improved appetite and growth despite pathogen exposure. This study demonstrates mixed probiotics could enhance the growth, immunity, and survival of Nile tilapia juveniles, highlighting their potential as sustainable prophylactics in aquaculture.

Key words: Aquaculture, *Oreochromis niloticus*, Probiotic, Immune modulation, Feed conversion rate

INTRODUCTION

The use of antibiotics in aquaculture has significantly improved the survival of cultured fish. However, it has also led to the emergence of antibiotic-resistance microbes, which pose a major threat to aquatic ecosystems and human health (Bondad-Reantaso et al. 2005; Primavera 2006). Residues of antibiotics can persist in the environment and enter the food chain, jeopardizing food safety and potentially causing harm to consumers over time (Alday et al. 2006). To address these challenges, a more sustainable approach is needed to reduce the dependency on antibiotics. This can be achieved by combining good husbandry practices with prophylactic agents, such as immuno-stimulants and probiotics (Naylor et al. 2000; Primavera 2006; Raza et al. 2024).

Probiotics have emerged as a promising, environmentally friendly alternative to improve fish health and growth (Hong et al. 2008; Michael et al. 2014). These beneficial microorganisms living in host organisms may stimulate phagocytic activity, thereby enhancing the immune system and providing disease resistance (Nikoskelainen et al. 2003). Dietary supplementation with probiotics modulates the gut microbiota, providing

Cite This Article as: Tung PS, Mastor NNI, Salim G, Septriani NI and Ransangan J, 2026. Probiotic benefits in Nile tilapia (Oreochromis niloticus) aquaculture: growth, immunity, and pathogen resistance. International Journal of Veterinary Science 15(1): 1-9. https://doi.org/10.47278/journal.ijvs/2025.108

nutritional benefits and mitigating the adverse effects of antibiotics and drugs (Nikoskelainen et al. 2003). Probiotics have long been used as dietary supplements for humans and animals (Balcázar et al. 2006; Tuan et al. 2013). To function effectively, they must survive harsh gastrointestinal conditions- gastric acid and bile salts (Michael et al. 2014). In aquaculture, probiotics optimize gut microbiota in fish, boosting nutrient uptake and growth rate (Soccol et al. 2010). As a result, fish reach marketable size faster, enhancing both yield and product quality (Cruz et al. 2012).

Probiotics suppress gut pathogens by secreting antagonistic compounds and out-compete them for nutrients and attachment sites, thereby preserving native microbiota and bolstering host immunity (Tapia-Paniagua et al. 2010; Pandiyan et al. 2013; Zapata, 2013). Maintaining a balanced gut ecosystem is vital for innate immune function and infection resistance (Siddque et al. 2015). In fish larvae, which rely on non-specific defenses, probiotic supplementation markedly improves disease resistance and survival during outbreaks (Rengpipat et al. 1998; Talpur et al. 2012; Tuan et al. 2013). Moreover, probiotics enhance water quality by degrading organic matter, suppressing harmful bacteria and promoting beneficial microbial and algal communities in aquaculture systems (Balcázar et al. 2006; Liu et al. 2010; Soccol et al. 2010).

Administered orally or via immersion, probiotics can establish long-term colonization in the teleost gut, ensuring sustained health and growth benefits (Cruz et al. 2012; Tuan et al. 2013). They stimulate leukocyte activity-elevating lymphocytes, neutrophils, and monocytes count-to strengthen innate defenses, suppress pathogens and poster beneficial microbes (Picchietti et al. 2007; Merrifield et al. 2010; Zokaeifar et al. 2012; Talpur et al. 2012; Pandiyan et al. 2013; Rungrassamee et al. 2014; Michael et al. 2014).

Blood parameters especially white blood cell (WBC) counts are fundamental indicators of fish health (Dal'Bó et al. 2015). Key leukocyte populations include neutrophils, monocytes (which differentiate into macrophages or dendritic cells), and lymphocytes, each orchestrating distinct defensive mechanisms (Tavares-Dias et al. 2011). Upon pathogens challenge, circulating WBCs increase: neutrophils rapidly home to infection sites, release granule proteins to modulate inflammation, and phagocytose invaders with enzymes and antimicrobial peptides (Lieschke and Trede 2009; Neumann et al. 2001). Monocyte-derived macrophages and dendritic cells then present antigens to lymphocytes, driving interleukins secretion and antibody production for adaptive immunity (Lieschke and Trede 2009).

This study evaluates three intestinal probiotic strains-*Shewanella algae*, *Enterobacter cloacae* and *Bacillus thuringiensis*, isolated from intestines of Sagor catfish (*Hexanematichthys sagor*) and goldfish (*Carassius auratus*). Administrated alone or in combination to tilapia juveniles challenged with a bacterial pathogen, these probiotics were assessed for their effects on growth rate, survival and WBC-driven immune responses.

MATERIALS AND METHODS

Experimental design and LD_{50} of bacterial pathogens

A total of 240 Nile tilapia (*Oreochromis niloticus*) juveniles, averaging 5±1g in weight, were divided across 24 aquaria in a closed aerated system with 10 fish per tank. The *Aeromonas hydrophila* pathogen stock was provided by the late Prof. Dr. Kishio Hatai and cultured in seven tubes over 24h according to Pang et al. (2019). Until each tube reached optical densities (OD_{600}) of 0.5, 0.8, 1.1, 1.3, 1.5, 1.8, and 2.0, respectively. Upon reaching the desired OD, the incubation of the particular tube was halted and stored for later use. Then each of the tubes was exposed to fish in triplicate tanks (21 fish per OD) via immersion as described by Feliatra et al. (2018), with three tanks serving as negative controls. The fish were observed daily for mortality and behavioural changes, or physical abnormalities. Uneaten feed and faeces were siphoned off each tank immediately after observation. On the 14th day, bacterial concentration in the water of each tank was quantified using serial dilution and presented in CFU/mL. The LD_{50} of the pathogen was calculated based on Reed and Muench's (1938) formula as stated in Equations 1 and 2:

$$\text{Proportionate distance} = \frac{\text{mortality above 50\%} - 50\%}{\text{mortality above 50\%} - \text{mortality below 50\%}}$$
(Equation 1)

$$LD_{50} = \text{Antilog (dilution above 50\% - proportionate distance)}$$
(Equation 2)

Preparation of probiotics

Probiotic candidates, isolated previously from the intestines of *Hexanematichthys sagor* (Pang et al. 2019) and *Carasius auratus* (Pang et al. 2020), were cultured in tryptic soy broth (TSB), and stored at 4°C. Before application, 2µL of each isolate was transferred to fresh tryptic soy agar (TSA) and incubated overnight at 27°C for 24-48h to allow the growth of the potential probiotic colonies of *Sh. algae*, *Ent. cloacae* and *B. thuringiensis* (Cowan and Steel 1961). The selected probiotic colonies were then picked using a rod and cultured in 10mL TSB for later use. Before application, each of the isolated stock was transferred to fresh TSB (10mL) and incubated overnight at 27ºC. Probiotic concentrations were standardized to an OD of 0.2 at 600nm using an Implen OD_{600} spectrophotometer (Implen Gmbh, Germany). Probiotic mixtures were prepared in triplicate based on predetermined proportions as stated in Table 1.

Table 1: The mixture, proportion, and volume of probiotic candidates

Mixture	Probiotic candidates	Proportion	Volume(mL)
A	*Shewanella algae*	1	18
B	*Enterobacter cloacae*	1	18
C	*Bacillus thuringiensis*	1	18
AB	*Sh. algae* and *Ent. cloacae*	1:1	9:9
AC	*Sh. algae* and *B. thuringiensis*	1:1	9:9
ABC	*Sh. algae*, *Ent. cloacae* and *B. thuringiensis*	1:1:1	6:6:6

Preparation of probiotic-treated feed

Probiotics were orally administered to tilapia juveniles by mixing with commercial fish feed. A total of 12g of the feed was prepared daily, sterilizing it on a tray with UV light. Then, the probiotic cultures (18mL) were evenly sprayed onto the feed, flipped twice to ensure uniform coating, and dried for 30 minutes. Treated feeds were stored at 4°C and weighed before and after feeding sessions. Unused feeds were discarded after the second daily feeding session. This process was repeated daily throughout the 14-day experimental period. A total of 170 tilapia juveniles (average weight 4.9±1g) were acclimatized for two weeks in a hatchery condition. The fish were fed with the probiotic-coated feed twice daily. After acclimatization, fish were redistributed into 10L aquaria (10 fish each). The fish were fed ad libitum, and uneaten feed and faeces were siphoned off daily. A closed, aerated system was maintained throughout the experiment. Probiotic treatments were performed either alone (*Sh. algae* (A), *Ent. cloacae* (B), and *B. thuringiensis* (C) or in combinations (e.g., AB for *Sh. algae* and *Ent. cloacae*). Probiotic concentrations were set to an OD_{600} of 0.2, corresponding to $6.7x10^7$CFU/mL for *Sh. algae*, $2.9x10^8$CFU/mL for *Ent. cloacae* and $6.2 x 10^7$CFU/mL for *B. thuringiensis*.

Pathogen exposure and experiment setup

Aeromonas hydrophila strain A10 (courtesy of Nagasaki University, Japan), was cultured in TSB and incubated at 27°C overnight. Pathogen suspensions at OD_{600} between 1.75 and 1.95 were freshly prepared for the *in vivo* challenge tests. Pathogen concentration for challenge tests was set at 1.43×10^8 CFU/mL. The pathogen was introduced to the tilapia juveniles on day 14 via immersion as described by Vijaybaskar and Somasundaram (2008).

Treatments included three controls and two sets of each probiotic combination (Table 2), all conducted in triplicate. Control 1 used untreated commercial feed with no pathogen (negative control). Control 2 used TSB-treated feed with no pathogen (negative probiotic control) and control 3 used TSB-treated feed with a pathogen (positive control).

Table 2: Experimental setup of probiotic fortified tilapia juveniles exposed to *Aeromonas hydrophila* strain A10

Treatments	Probiotics			TSB	Pathogen
	A	B	C		
Control 1	No	No	No	No	No
Control 2	No	No	No	Yes	No
Control 3	No	No	No	Yes	Yes
A1	Yes	No	No	Yes	No
A2	Yes	No	No	Yes	Yes
B1	No	Yes	No	Yes	No
B2	No	Yes	No	Yes	Yes
C1	No	No	Yes	Yes	No
C2	No	No	Yes	Yes	Yes
AB1	Yes	Yes	No	Yes	No
AB2	Yes	Yes	No	Yes	Yes
BC1	No	Yes	Yes	Yes	No
BC2	No	Yes	Yes	Yes	Yes
AC1	Yes	No	Yes	Yes	No
AC2	Yes	No	Yes	Yes	No
ABC1	Yes	Yes	Yes	Yes	No
ABC2	Yes	Yes	Yes	Yes	No

Note: A = *Sh. algae*; B = *Ent. cloacae*; C= *B. thuringiensis*; AB, AC, BC and ABC=Mixed probiotics

Growth measurement of fish

Fish length and weight were recorded on days 0, 7, 14, 21, and 28. Five fish per treatment group were randomly sampled for calculation of specific growth rates (SGR) as described by Crane et al. (2020) in Equation 3.

$$SGR= \frac{100\ (\text{In}\ W2-\text{In}\ W1)}{t2-t1} \qquad \text{Equation 3}$$

Where W_1 and W_2 are initial and final weights; t_1 and t_2 are time points.

Leucocyte count under pathogen challenge

The effect of probiotics on WBC (white blood cells) count, namely lymphocytes, neutrophils, and monocytes, was evaluated in tilapia juveniles challenged with the *A. hydrophila*. Blood was sampled as described by Argungu et al. (2016). Briefly, fish were sedated in aerated tanks with anesthetic solution (Nika Transmore, Sagar Aquarium, India), and 0.5 ml of blood was drawn from the caudal vein into EDTA-treated tubes before being smeared on a microscope slide. Blood smears were stained with Giemsa and analysed under a microscope. The WBC morphology was classified as per Claver and Quaglia (2009) and Clauss et al. (2008).

Statistical Analysis

Growth, feed utilization, feed conversion ratio (FCR), and WBC composition data were analysed using the SPSS version 2.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test, and non-normal data were log-transformed prior to one-way ANOVA analysis.

RESULTS

Lethal dose (LD_{50}) of *A. hydrophila*

As shown in Table 3, the LD_{50} for tilapia juveniles over a 14-day period was determined to be 8.13×10^{11} CFU/mL. This value corresponded to an optical density (OD_{600}) range of 1.7-2.0, where bacterial concentrations ranged from 9.7×10^9 to 5.3×10^{11} CFU/mL. The calculation followed the Reed and Muench (1938) method, utilising a proportionate distance of 0.91 to refine the LD_{50} estimate. Interestingly, all fish in control groups exhibited symptoms associated with Motile *Aeromonas Septicaemia* (MAS), such as necrosis, red sores, and haemorrhagic septicaemia, which agreed with the description of Pridgeon and Klesius (2011). These findings emphasized the virulence of *A. hydrophila* and its significant impact on tilapia health, even at lower OD_{600} levels.

Growth performance without pathogen challenge

Table 4 reveals notable growth in fish across all probiotic treatments without exposure to *A. hydrophila*. The initial weight of 4.99±0.03g had increased by 56.19% to 7.79 ± 0.10g by the end of the experiment. Fish length followed a similar growth trend, with the mean increasing by 50.86 % from 5.17±0.0362 cm to 7.79 ± 0.07cm. While no significant differences were observed between probiotic treatments and the control (T1), treatment A1 (*Sh. algae*) and ABC1 (a mixture of *Sh. algae, B. thuringiensis,* and *Ent. cloacae*) showed higher mean SGR values, with increases of 0.33% and 0.06%, respectively.

Growth performance under pathogen challenge

Table 5 shows the mean weight and length of probiotic-treated fish that were challenged with *A. hydrophilia*. In the pathogen-challenged fish, weights increased by 63.59%, from an initial average of 4.9774±0.03g to 8.1426±0.103g. Fish lengths increased by 60%, from 5.0417±0.028cm to 8.0670±0.088cm.

Table 3: LD_{50} of *Aeromonas hydrophila* A10 to tilapia juveniles recorded after 14 days

OD	CFU/mL	Initial number	Average mortality	Average survival	Death ratio	Survival ratio	Mortality	Cumulative mortality
2.0	10^{11}	10	8	2	25	5	25/30	83.33%
1.7	10^{9}	10	5	5	14	16	14/30	46.67%
1.5	10^{8}	10	4	6	13	17	13/30	43.33%
1.3	10^{7}	10	4	6	11	19	11/30	36.67%
1.0	10^{5}	10	2	8	7	23	7/30	23.33%
0.8	10^{4}	10	1	9	2	28	2/30	6.67%
0.5	10^{2}	10	0	10	0	30	0/30	0
0	0	10	0	10	0	30	0/30	0

Table 4: Mean weight and length of fish under various probiotic treatments without pathogen challenge

Treatments	Initial		Week 1		Week 2		Week 3		Week 4		Total Gain	
	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)
T1	4.83	5.19	5.81	6.29	6.65	6.91	7.16	7.44	8.01	7.82	3.18	2.63
T2	5.15	5.25	6.19	6.40	6.73	6.86	7.33	7.38	8.04	7.95	2.89	2.70
A1	4.87	5.19	6.26	6.45	6.28	6.90	7.26	7.47	8.03	7.75	3.17	2.55
B1	5.22	5.91	6.60	6.98	6.65	6.90	7.14	7.25	7.62	7.56	2.40	1.65
C1	4.98	5.07	6.13	6.27	6.67	6.79	6.89	7.08	7.39	7.45	2.41	2.38
AB1	4.88	4.94	5.75	5.74	6.33	6.49	7.19	7.24	7.76	7.87	2.88	2.93
AC1	5.05	5.07	5.83	5.97	6.33	6.39	7.05	7.25	7.81	8.04	2.77	2.97
BC1	4.95	4.99	5.57	5.67	6.45	6.61	7.15	7.45	7.91	8.19	2.96	3.19
ABC1	4.97	4.91	6.11	6.11	6.35	6.38	6.75	6.69	7.53	7.52	2.56	2.61

Note: W=Total weight; L=Standard length; T= Control; A = Probiotic *Sh. algae*; B = Probiotic *Ent. cloacae*; C= Probiotic *B. thuringiensis*; AB, AC, BC and ABC = mixed probiotics

Table 5: Mean weight and length of fish in probiotic treatment with pathogen challenge

Treatments	Initial		Week 1		Week 2		Week 3		Week 4		Total Gain	
	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)	W(g)	L(cm)
T3	5.07	5.13	6.57	6.65	7.12	7.10	7.45	7.45	8.01	7.83	2.95	2.70
A2	4.71	5.01	5.89	6.37	6.73	7.06	6.73	7.26	7.81	7.74	3.09	2.73
B2	5.08	5.13	7.09	7.14	7.38	7.49	7.98	7.87	8.94	8.57	3.86	3.44
C2	5.05	5.11	6.29	6.43	6.79	7.00	7.29	7.33	7.75	7.79	2.69	2.68
AB2	4.99	5.05	5.64	5.72	6.61	6.67	7.81	7.69	8.78	8.82	3.79	3.77
AC2	4.84	4.84	5.79	5.71	6.73	6.74	7.74	7.81	8.69	8.81	3.85	3.97
BC2	5.11	5.02	6.37	6.25	6.47	6.70	7.06	7.03	7.57	7.70	2.46	2.68
ABC2	5.03	5.03	5.93	5.95	6.33	6.45	7.01	6.95	7.45	7.19	2.43	2.16

Note: W=Total weight; L=Standard length; T= Control; A = Probiotic *Sh. algae*; B = Probiotic *Ent. cloacae*; C= Probiotic *B. thuringiensis*; AB, AC, BC and ABC = Mixed probiotics

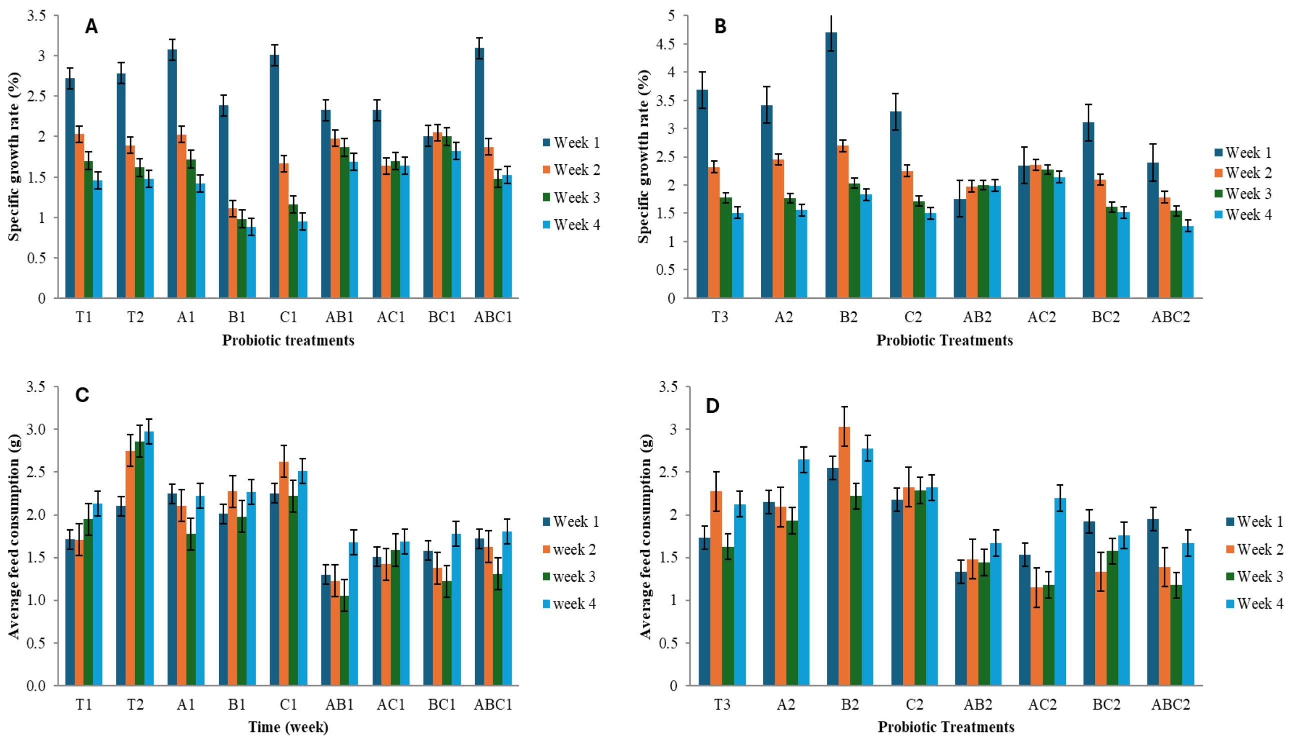

Fig. 1: Specific growth rate of probiotic-treated fish without (A) and with (B) pathogen challenge and the average feed consumption of probiotic-treated fish without (C) and with (D) pathogen challenge.

Weight and length increments were also consistent across the range of fish sizes (4.10-6.00g to 5.50-10.70g and 4.30-5.50cm to 5.70-10.40cm, respectively). Despite these gains, the SGR of fish across all probiotic treatments did not significantly differ from control treatment T3 (P>0.05). However, fish in treatment B2 (*E. cloacae*) demonstrated a 1.98±0.747 higher SGR than controls, indicating a potential benefit under pathogen pressure. Fig. 1A and 1B show the distribution of specific growth rate of probiotic-treated tilapia over four weeks with and without pathogen challenge. Meanwhile, Fig. 1C and 1D show the average feed consumption of probiotic-treated fish with and without pathogen challenge.

White blood cell counts

Fig. 2 shows Giemsa staining of the blood smear for the tilapia in this study. Fish without probiotic treatment had the highest leukocyte count (4465.00±1042.75 cells/µL), followed by those treated with one type (2681.83±457.36 cells/µL), two types (1946.83±599.45 cells/µL), and three types of probiotics (1441.50±109.84 cells/µL), respectively. Interestingly, fish exposed to pathogens had slightly lower leukocyte counts (5034.56±1144.47 cells/µL) than unexposed fish (5500.16±1064.92 cells/µL). Fig. 3 shows comparisons of the average white blood counts in prebiotic-treated fish that had and had not been exposed to *A. hydrophilia*.

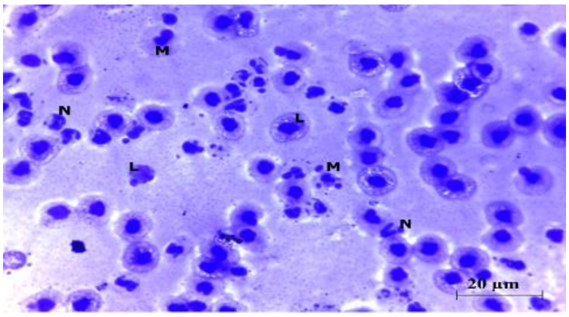

Fig. 2: Fish leukocytes under light microscope with 100x magnification. Note: Lymphocyte (L); neutrophil (N) and monocyte (M).

In fish that were unexposed to pathogens, the lymphocyte count in treatment ABC (*Sh. algae*, *B. thuringiensis*, and *Ent. cloacae*) was significantly lower than in control treatment T1 (P < 0.05). Notably, only T2 (TSB-sprayed feed) showed a slight increase in lymphocyte count compared to T1. Under pathogen challenge, no significant differences were observed between treatments and controls (T3). However, treatments C2 (*B. thuringiensis*), AB2 (*Sh. algae* and *Ent. cloacae*), and ABC2 exhibited marginally lower lymphocyte counts than controls.

Neutrophil counts

Neutrophil count in unchallenged fish was significantly lower in treatment ABC1 compared to control T1 (P<0.05), while T2 and C1 (*B. thuringiensis*) showed slightly higher counts than T1. In pathogen-challenged fish, neutrophil counts were significantly lower in treatment AB2 than in controls (T3, P<0.05), with all other probiotic treatments also showing lower neutrophil counts relative to controls.

Monocyte counts

Monocyte counts, analysed via Tukey's post hoc test, were generally not significantly different across treatments, whether the fish were pathogen-challenged or not. However, in unchallenged fish, T2 had slightly higher monocyte counts than T1. Under pathogen challenge, treatment ABC2 showed marginally higher monocyte counts than T3, though the differences were not statistically significant.

Survival rate of fish

Survival rates across all treatments were 100%, irrespective of pathogen challenge. However, some fish in pathogen-exposed treatments exhibited clinical signs of infection, such as tail rot and skin necrosis (Fig. 4). This suggests that while probiotics might not significantly protect the fish under pathogen pressure, they could mitigate disease severity.

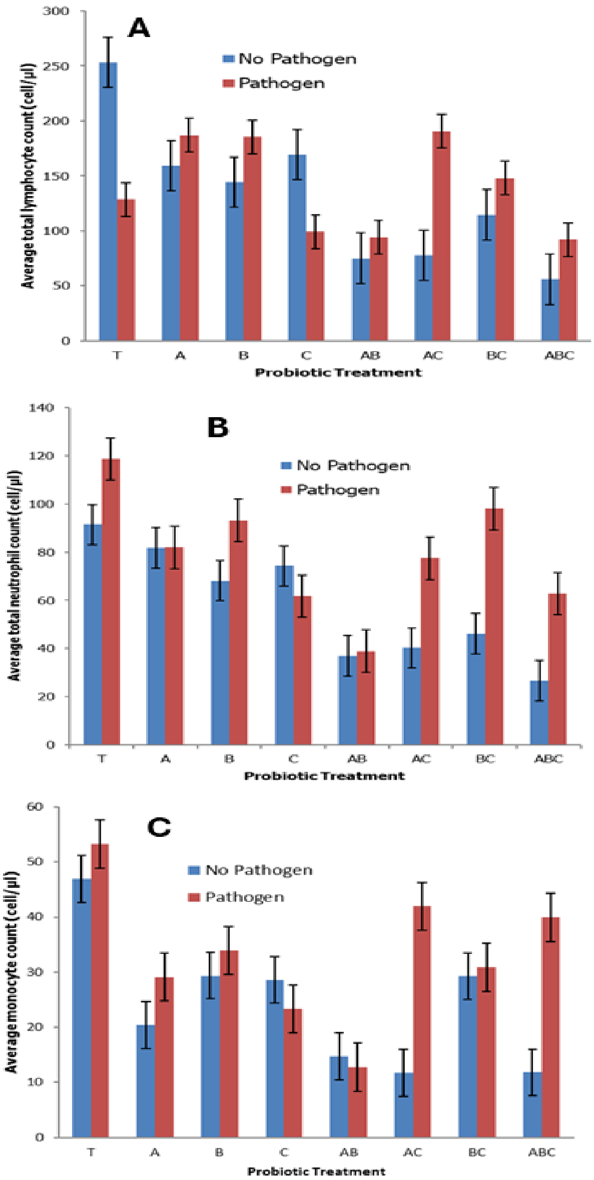

Fig. 3: Average white blood cell counts of various-treated tilapia fish with and without pathogen challenge. The cells comprise lymphocytes (A), neutrophils (B), and monocytes (C).

DISCUSSION

The determination of LD_{50} provided critical insights into the pathogenicity of *A. hydrophila* and its effects on fish health. Various methods, including oral administration, injection, and bath immersion, have been employed to introduce pathogens (Feliatra et al. 2018) or chemicals to host organisms. Among these, bath immersion was particularly suitable for aquatic animals because it mimicked a natural pathogen exposure in aquaculture systems (Brake et al. 2017). This method aligned closely with real-world conditions, where pathogens are mostly introduced via water, making it more ecologically relevant (Vaseeharan et al. 2004).

Fig. 4: Tilapia with signs of *A. hydrophilia* infection (tail rot and skin necrosis).

In this study, no mortality in tilapia hosts was observed, even when the fish were exposed to the LD_{50} of *A. hydrophila*. This finding highlighted the importance of external and internal factors in determining LD_{50}, such as fish size, age, health status, and environmental conditions (Kroupova et al. 2005). For instance, fish used in LD_{50} experiments were lighter and potentially less resistant than those used in subsequent in vivo pathogen challenges, as larger fish typically exhibited greater disease resistance (Haniffa et al. 2015). These variables emphasize the complexity of assessing pathogen lethality and suggest that standardized experimental designs are necessary for a more precise evaluation.

Pathogen exposure could significantly impact fish appetite and growth (Manchanayake et al. 2023). Diseased fish often exhibited reduced feeding behaviour, leading to slower growth rates. Probiotics could mitigate these effects by improving nutrient absorption and digestion, even under pathogen-induced stress. In this study, tilapia treated with a combination of probiotics exhibited varying feeding responses, with some treatments showing reduced feed consumption compared to controls.

Interestingly, fish treated with combinations of *Sh. algae* (probiotic A), *Ent. cloacae* (probiotic B), and *B. thuringiensis* (probiotic C) consumed less feed but could maintain similar growth rates as others. This finding supported the hypothesis that probiotics could enhance feed efficiency by breaking down complex nutrients into smaller, more absorbable particles (Balcázar et al. 2006). The ability of probiotics to produce extracellular enzymes (Cruz et al. 2012), such as proteases and lipases (Yi et al., 2020; Jamil et al. 2023), might aid in nutrient assimilation, promoting growth even when feed intake was reduced. However, the lower appetite observed in treatments involving *Sh. algae* could be attributed to the novelty of this marine-origin probiotic (Uniacke-Lowe et al. 2024) to freshwater tilapia. Despite reduced feed consumption, the specific growth rate (SGR) of fish in *Sh. algae* treatments were not significantly different from other treatments, indicating their potential to improve feed utilization and growth.

Probiotic treatments were observed to influence growth performance differently under pathogen-free and pathogen-challenged conditions. Fish treated with *Ent. cloacae* (probiotic B) exhibited notable growth improvements, with higher SGRs under both conditions. The growth-enhancing properties of *Ent. cloacae* could be attributed to its enzymatic activity, particularly in fermenting carbohydrates and producing bioactive compounds that promoted gut health (Fajingbesi et al. 2018; Yi et al. 2020). Moreover, the combination of *Ent. cloacae* with other probiotics, such as *B. thuringiensis*, further enhanced growth by synergistically improving nutrient digestibility (Cruz et al. 2012; Galli et al. 2024). These findings were in line with previous research that suggests probiotic mixtures can optimize enzymatic activity and gut microbiota balance (Torres-Maravilla et al. 2024), thereby supporting host growth even under pathogen stress (Capkin and Altinok 2008).

The immune-modulating effects of probiotics were evident in this study, as fish treated with probiotics showed variations in leukocyte counts under pathogen challenge. Leukocyte counts, particularly lymphocytes, neutrophils, and monocytes, serve as key indicators of immune response (Mokhtar et al. 2023). When invaded by a pathogen, the host immune system would be triggered, and this would increase the leukocyte count (Adeyemi et al. 2013). Therefore, the reduction in leukocyte counts observed in fish treated with mixed probiotics suggest that the probiotic treatment could modulate the immune system (Ridha and Azad 2012; Gewaily et al. 2021) by reducing stress-induced inflammation.

Probiotic treatments, especially those involving *Sh. algae* and *B. thuringiensis*, also appeared to lower cortisol levels (Rollo et al. 2006; Wuertz et al. 2021), a stress hormone known to suppress lymphocyte production (Davis et al. 2008). Reduced lymphocyte counts (Sîrbu et al. 2022) in probiotic-treated fish might reflect a balance between immune activation and regulation, preventing excessive inflammatory responses that could impair growth and health.

Additionally, probiotics could also enhance the innate immune system by stimulating cellular components such as neutrophils and macrophages (Nayak 2010; Adejumo et al. 2023), which would migrate to infection sites to carry out phagocytosis (Fachri et al. 2024). This mechanism aligned with the dual role of probiotics in activating the innate immune system and priming adaptive immunity through antigen presentation and antibody production (Zhang et al. 2024).

The feed conversion ratio (FCR) is a critical metric for evaluating feeding efficiency in aquaculture. Lower FCR values indicated better conversion of feed into biomass. In this study, fish treated with mixed probiotics demonstrated lower FCR values compared to controls, suggesting enhanced feed utilization (El-Saadony et al. 2021; Torres-Maravilla et al. 2024). The ability of probiotics to improve gut microbiota composition and enzymatic activity likely contributed to this efficiency (Balcázar et al. 2006; Yi et al. 2020; El-Saadony et al. 2021).

Interestingly, fish treated with mixed probiotics were also observed to achieve comparable or superior growth rates despite consuming less feed than those treated with a single probiotic and controls. This highlights the potential cost-saving benefits of probiotic supplementation in aquaculture, as reduced feed consumption could lower production costs without compromising growth.

Despite pathogen exposure, no fish mortality was observed throughout the study period. This could be attributed to the robust health status of the fish as well as the protective effects of probiotics. Probiotic treatments, particularly those involving *Ent. cloacae* and *B. thuringiensis*, could enhance survival by improving gut health (Girijakumari et al. 2018) and reducing pathogen colonization (Dhayalan et al. 2025). The production of antimicrobial compounds by probiotics (Girijakumari et al. 2018; Fijan 2023) likely inhibited pathogen growth, minimising the severity of infections. However, some fish in pathogen-challenged treatments exhibited signs of Motile Aeromonas Septicemia (MAS), such as tail rot and skin necrosis. These symptoms emphasize the virulence of *A. hydrophila* and the need for vigorous integrated disease management strategies in aquaculture facilities including the use of prebiotics and vaccines.

Conclusion

This study highlighted the many benefits of probiotics in aquaculture, demonstrating their potential to enhance growth, feed efficiency, immune modulation, and pathogen resistance in tilapia. The use of combined probiotics such as *Sh. algae*, *E. cloacae*, and *B. thuringiensis*, could improve nutrient absorption, reduced FCR, and support fish resistance towards a pathogen. Probiotics could promote gut health through enzymatic activity, balanced immune response, and suppression of pathogen colonisation. Despite no mortality were seen in pathogen-challenged fish, symptoms like tail rot and skin necrosis were present, and this emphasizes the need for a more vigorous integrated management strategy in aquaculture. Notably, marine-origin probiotics like *Sh. algae* demonstrated beneficial to freshwater fish, suggesting its application for cross-environments. Future research should focus on optimising probiotic combinations and exploring their long-term effects on stress reduction. Overall, the use of probiotics presents a promising, cost-effective solution for improving aquaculture productivity and fish welfare while reducing reliance on antibiotics and chemicals.

DECLARATIONS

Funding: This study was funded by the Ministry of Higher Education Malaysia under the Higher Institution Centre of Excellence (HICoE) Research Grant No. CEO0007.

Acknowledgement: The authors would like to thank the Ministry of Higher Education Malaysia for providing research fund to carry out this study.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: All the data generated during the study are presented in the article.

Ethics Statement: The experimental procedures conducted in this study were reviewed and approved by the Universiti Malaysia Sabah Animal Care and Use Committee (UMS ACUC) under approval letter No. AEC0013/2024.

Author's Contribution: PST carried out the study, data analysis, manuscript drafting, and administration of the project; NNIM reviewed and edited the draft manuscript; GS reviewed and edited the draft manuscript. NIS reviewed and edited the draft manuscript; JR developed research idea, secured the research grant, administered the project, reviewed and finalized the manuscript for submission.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Adejumo SA, Oli AN, Rowaiye AB, Igbokwe NH, Ezejiegu CK and Yahaya ZS, 2023. Immunomodulatory benefits of probiotic bacteria: A review of evidence. OBM Genetics 7(4): 1-73. https://doi.org/10.21926/obm.genet.2304206

Adeyemi JA, Atere TG, Oyedara OO, Olabiyi KO and Olaniya OO, 2013. Hematological assessment of health status of African catfish *Clarias gariepinus* (Burchell 1822) experimentally challenged with *Escherichia coli* and *Vibrio fischeri*. Comparative Clinical Pathology 23: 1309-1313. https://doi.org/ 10.1007/s00580-013-1780-y

Alday V, Guichard B, Smith P and Uhland C, 2006. Joint FAO / WHO / OIE. Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance Towards a risk analysis of antimicrobial use in aquaculture, pp: 29.

Argungu LA, Siraj SS, Christianus A, Amin MSN, Daud SK, Abubakar, MS, Abubakar IA and Aliyu-Paiko M, 2016. A simple and rapid method for blood collection from walking catfish, *Clarias batrachus* (Linneaus, 1758). Iranian Journal of Fisheries Sciences 16 (3): 935-944.

Balcázar JL, Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D and Muzquiz JL, 2006. The role of probiotics in aquaculture. Veterinary Microbiology 114: 173-186. https://doi.org/10.1016/j.vetmic.2006.01.009

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Tan Z and Shariff M, 2005. Disease and health management in Asian aquaculture. Veterinary Parasitology 132(3-4): 249-272. https://doi.org/10.1016/j.vetpar.2005.07.005

Brake K, Gumireddy A, Tiwaari M, Chauhan H and Kumari D, 2017. In vivo studies for drug development via oral delivery: Challenges, animal models and techniques. Pharmaceutica Analytica Acta 8(9): 1-11. https://doi.org/10.4172/2153-2435.1000560

Capkin E and Altinok I, 2008. Effects of dietary probiotic supplementations on prevention/treatment of yersiniosis diseases. Journal of Applied Microbiology 106: 1147-1153. https://doi.org/10.1111/j.1365-2672.2008.04080.x

Clauss TM, Dove ADM and Arnorld JE, 2008. Hematologic disorder of fish. Veterinary Clinics of North America 11:

445-462. https://doi.org/10.1016/j.cvex.2008.03.007

Claver JA and Quaglia AIE, 2009. Comparative morphology, development, and function of blood cells in nonmammalian vertebrates. Journal of Exotic Pet Medicine 18 (2): 87-97. https://doi.org/10.1053/j.jepm.2009.04.006

Cowan ST and Steel KJ, 1961. Diagnostic tables for the common medical bacteria. Journal of Hygiene 59(3): 357-372. https://doi.org/10.1017/s0022172400039024

Cruz PM, Ibanez AL, Hermosillo OAM and Ramirez Saad HC, 2012. Use of probiotics in aquaculture. Microbiology 916845: https://doi.org/10.5402/2012/916845

Dal'Bó GA, Sampaio FG, Losekann ME, de Queiroz JF, Baretto Luiz AJ, Wolf VHG, Gonçalvess VT and Carra ML, 2015. Hematological and morphometric blood value of four cultured species of economically important tropical foodfish. Neutrophical Ichthyology 13(2): 439-446. https://doi.org/10.1590/1982-0224-20140115

Davis AK, Maney DL and Maerz JC, 2008. The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Functional Ecology 22: 760-772. https://doi.org/10.1111/j.1365-2435.2008.01467.x

Dhayalan A, Kamala Jayanthi PD, Manoharan S, Nadeem A, Govindasamy B, Pachiappan P and Vasudhevan P, 2025. Fish gut symbiotic bacterium *Bacillus thuringiensis*: RSM optimization for its extracellular lipase enzyme production, lipase-protein purification, characterization, and docking analysis. International Journal of Biological Macromolecules 301: 140428. https://doi.org/10.1016/j.ijbiomac.2025.140428

El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MAO, Dhama K, Abdel-Latif HMR, 2021. The functionality of probiotics in aquaculture: An overview. Fish and Shellfish Immunology 117: 36–52. https://doi.org/10.1016/j.fsi.2021.07.007

Fachri M, Amoah K, Huang Y, Cai J, Alfatat A, Ndandala CB, Shija VM, Jin X, Bissih F and Chen H, 2024. Probiotics and paraprobiotics in aquaculture: a sustainable strategy for enhancing fish growth, health and disease prevention-a review. Frontiers in Marine Science 11:1499228. https://doi.org/10.3389/fmars.2024.1499228

Fajingbesi AO, Anzaku AA, Akande M, Emmanuel IA and Akwashiki O, 2018. Production of protease enzyme from fish gut using *Pseudomonas fluorescens*, *Enterobacter cloacae* and *Bacillus megaterium*. Journal of Clinical Pathology and Laboratory Medicine 2: 1-7.

Feliatra F, Elizal E, Lukistyowati I, Melina D and Ramadhani M, 2018. Effectiveness of immersion with probiotic in improving health of Nile tilapia (*Oreochromis niloticus*). Asian Journal of Animal and Veterinary Advances 13: 43-51. https://doi.org/10.3923/ajava.2018.43.51

Fijan S, 2023. Probiotics and their antimicrobial effect. Microorganisms, 11: 528. https://doi.org/10.3390/microorganisms11020528528

Galli GM, Andretta I, Levesque C, Stefanello T, Carvalho CL, Perez Pelencia JY, Bueno Martins G, Souza de Lima Cony B, Romeiro de Oliveira C, Franceschi CH, Kipper M, 2024. Using probiotics to improve nutrient digestibility and gut-health of weaned pigs: A comparison of maternal and nursery supplementation strategies. Frontiers in Veterinary Science 11: 1356455. https://doi.org/10.3389/fvets.2024.1356455

Gewaily MS, Shukry M, Abdel-Kader MF, Alkafafy M, Farrag FA, Moustafa EM, Doan HV, Abd-Elghany MF, Abdelhamid AF, Eltanahy A and Dawood MAO, 2021. Dietary *Lactobacillus plantarum* relieves Nile tilapia (*Oreochromis niloticus*) juvenile from oxidative stress, immunosuppression, and inflammation induced by deltamethrin and *Aeromonas hydrophila*. Frontiers in Marine Science 8:621558. https://doi.org/10.3389/fmars.2021.621558

Girijakumari NR, Ethiraja K and Marimuthu PN, 2018. In vitro and in vivo evaluation of probiotic properties of *Enterobacter cloacae* in Kenyi cichlid, *Maylandia lombardoi.* Aquaculture International 26: 959-980. https://doi.org/10.1007/s10499-018-0262-2

Haniffa MA, Ramakrishnaan M and Sheela PAJ, 2015. Investigation on virulence dose and antagonistic activity of selected probiotics against *Aphanomyces invadans* and *Aeromonas hydrophila*. International Journal of Pharmacy and Pharmaceutical 2: 53-65.

Hong HA, Huang JM, Khaneja R, Hiep LV, Urdaci MC and Cutting SM, 2008. The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics. Journal of Applied Microbiology 105(2): 510–520. https://doi.org/10.1111/j.1365-2672.2008.03773.x

Jamil R, CR Wei, MRJ Khan, AMA Khan, M Faisal, M Awais, MI Asghar & UB Tahir, 2023. Integration of plant-based proteins into aquaculture diets for sustainable fish growth and wellness. In: Complementary and Alternative Medicine: One Health Perspective, FahumSci, Lahore, Pakistan, pp: 204-214. https://doi.org/10.61748/CAM.2023/028

Kroupova H, Machova J and Svobodova Z, 2005. Nitrite influence on fish: A review. Veterinarni Medicina 50(11): 461–471. http://dx.doi.org/10.17221/5650-VETMED

Lieschke GJ and Trede NS, 2009. Fish immunology. Current Biology 19(16): R678

Liu K, Chiu C, Shiu Y, Cheng W and Liu C, 2010. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish and Shellfish Immunology 28: 837-844. https://doi.org/10.1016/j.fsi.2010.01.012

Manchanayake T, Salleh A, Mohammad NAA, Ina Salwany MY and Zamri SM, 2023. Pathology and pathogenesis of *Vibrio* infection in fish: a review. Aquaculture Reports 28: 101459. https://doi.org/10.1016/j.aqrep.2022.101459

Crane DP, Ogle DH and Shoup DE, 2020. Use and misuse of a common growth metric: guidance for appropriately calculating and reporting specific growth rate. Reviews in Aquaculture 12: 1542-1547. https://doi.org/10.1111/raq.12396

Merrifield DL, Dimitroglou A, Foey A, Davies SJ, Baker RTM, Bøgwald J, Castex M and Ringø E, 2010. The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture 302(1–2): 1–18. https://doi.org/10.1016/j.aquaculture.2010.02.007

Michael ET, Amos SO and Hussaini LT, 2014. A review on probiotics application in aquaculture. Fisheries and Aquaculture Journal 5: 111. https://doi.org/10.4172/2150-3508.1000111

Mokhtar DM, Zaccone G, Alesci A, Kuciel M, Hussein MT and Sayed RKA, 2023. Main components of fish immunity: An overview of the fish immune system. Fishes 8: 93. https://doi.org/10.3390/fishes8020093

Nayak SK, 2010. Probiotics and immunity: A fish perspective. Fish and Shellfish Immunology, 29: 2-14. https://doi.org/10.1016/j.fsi.2010.02.017

Naylor R, Goldburg R, Primavera J, Kautsky N, Beveridge M, Clay J, Folke C, Lubchenco J, Mooney, H and Troell M, 2000. Effect of aquaculture on world fish supplies. Nature 405: 1017–1024. https://doi.org/10.1038/35016500

Neumann NF, Stafford JL, Barreda, D, Ainsworth AJ and Belosevic, M, 2001. Antimicrobial mechanisms of fish phagocytes and their role in host defense. Developmental and Comparative Immunology 25(8-9): 807-825. https://doi.org/10.1016/S0145-305X(01)00037-4

Nikoskelainen S, Ouwehand AC, Bylund G, Salminen S and Lilius E, 2003. Immune enhancement in rainbow trout (*Oncorhynchus mykiss*) by potential probiotic bacteria (*Lactobaciluus rhamnosus*). Fish and Shellfish Immunology

15: 443-452. https://doi.org/10.1016/s1050-4648(03)00023-8
Pandiyan P, Balaraman D, Thirunavukkarasu R, George EGJ, Subaramaniyan K, Manikkam S and Sadayappan B, 2013. Probiotics in aquaculture. Drug Invention Today 5(1): 55–59. https://doi.org/10.1016/j.dit.2013.03.003
Pang ST, Ransangan J and Hatai K, 2020. Isolation, identification and preliminary characterization of candidate probiotic bacteria from the intestine of domesticated goldfish (*Carassius auratus*). Journal of Fisheries and Environment 44(2): 39-52. https://li01.tci-thaijo.org/index.php/JFE/article/view/209494
Pang ST, Ransangan J and Hatai K, 2019. Isolation, growth profile and probiotic properties of autochthonous intestinal bacteria of sagor catfish (*Hexanematichthys sagor*). Biocontrol Science 24(1): 1-11. http://dx.doi.org/10.4265/bio.24.1
Picchietti S, Mazzini M, Taddei AR, Renna R, Fausto AM, Mulero V, Carnevali O, Cresci A and Abelli L, 2007. Effects of administration of probiotic strains on GALT of larval gilthead seabream: Immunohistochemical and ultrastructural studies. Fish and Shellfish Immunology 22(1): 57–67. https://doi.org/10.1016/j.fsi.2006.03.009
Pridgeon JW and Klesius PH, 2011. Virulence of *Aeromonas hydrophila* to channel catfish *Ictaluras punctatus* fingerlings in the presence and absence of bacterial extracellular products. Diseases of Aquatic Organisms 95: 209-215. https://doi.org/10.3354/dao02357
Primavera J, 2006. Overcoming the impacts of aquaculture on the coastal zone. Ocean and Coastal Management 49(9-10): 531-545. https://doi.org/10.1016/j.ocecoaman.2006.06.018
Raza A, Abbas RZ, Karadağoğlu Ö, Raheem A, Khan AMA, Khalil MZ, Maheen N, Quddus A, Hussain A and Kanchev KP, 2024. Role of probiotics in increasing meat and egg production in poultry: A review. Kafkas Universitesi Veteriner Fakultesi Dergisi 30 (6): 753-760. https://doi.org/10.9775/kvfd.2024.32861
Reed LJ and Muench H, 1938. A simple method of estimating fifty percent endpoints. American Journal of Epidemiology 27(3): 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408
Rengpipat S, Phianphak W, Piyatiratitivorakul S and Menasveta P, 1998. Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture 167(3–4): 301–313. https://doi.org/10.1016/S0044-8486(98)00305-6
Ridha MT and Azad IS, 2012. Preliminary evaluation of growth performance and immune response of Nile tilapia *Oreochromis niloticus* supplemented with two putative probiotic bacteria. Aquaculture Research 43: 843–852. https://doi.org/10.1111/j.1365-2109.2011.02899.x
Rollo A, Sulpizio R, Nardi M, Silvi S, Orpianesi C, Caggiano M, Cresci A and Carnevali O, 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiology and Biochemistry 32: 167–177. https://doi.org/10.1007/s10695-006-0009-2
Rungrassamee W, Klanchui A, Maibunkaew S, Chaiyapechara S, Jiravanichpaisal P and Karoonuthaisiri N, 2014. Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*). PLOS 9(3): 1–13. https://doi.org/10.1371/journal.pone.0091853
Siddque MAB, Sarker NR, Hamid MA, Amin MN and Sultana M, 2015. Growth performance, feed conversion ratio and economics of production of native and crossbred (Local x Holstein Friesian) bulls for fattening under different improved feeding. Journal of Agricultural Science and Technology 5: 770-780. http://dx.doi.org/10.17265/2161-6256/2015.09.008
Sîrbu E, Dima MF, Tenciu M, Cretu M, Coada MT, Totoiu A, Cristea V and Patriche N, 2022. Effects of dietary supplementation with probiotics and prebiotics on growth, physiological condition, and resistance to pathogens challenge in Nile tilapia (*Oreochromis niloticus*). Fishes 7: 273. https://doi.org/10.3390/fishes7050273
Soccol CR, Vandenberghe LPS, Spier MR, Medeiross BP, Yamaguishi CT, Lindner JD, Pandey A and Thomaz-Soccol V, 2010. The potential of probiotics. A review. Food Technology and Biotechnology 48 (4): 413-434.
Talpur AD, Memon AJ, Khan MI, Ikhwanuddin M, Daniel M, Danish M and Abol-Munafi AB, 2012. Inhibition of pathogens by lactic acid bacteria and application as water additive multi isolates in early stages larviculture of *P. pelagicus* (Linnaeus, 1758). Journal of Animal and Plant Sciences 22(1): 54–64.
Tapia-Paniagua ST, Chabrillión M and Díaz-Rosales P, 2010. Intestinal microbiota diversity of the flat fish *Solea senegalensis* (Kaup, 1858) following probiotic administration. Microbial Ecology 60: 310-319. https://doi.org/10.1007/s00248-010-9680-z
Tavares-Dias M, Monteiro AMC, Affonso EG and Amaral KDS, 2011. Weight-length relationship, condition factor and blood parameters of farmed *Cichla temensis* Humboldt, 1821 (Cichlidae) in central Amazon. Neotrophical Ichthyology 9(1): 113-119. https://doi.org/10.1590/S1679-62252011005000010
Torres-Maravilla E, Parra M, Maisey K, Vargas RA, Cabezas-Cruz A, Gonzalez A, Tello M and Bermúdez-Humarán LG, 2024. Importance of probiotics in fish aquaculture: Towards the identification and design of novel probiotics. Microorganisms 12: 626. https://doi.org/10.3390/microorganisms12030626
Tuan TN, Duc PM and Hatai K, 2013. Overview of the use of probiotics in aquaculture. International Journal of Research in Fisheries and Aquaculture 3(3): 89-97.
Uniacke-Lowe S, Stanton C, Hill C and Ross P, 2024. The marine fish gut microbiome as a source of novel bacteriocins. Microorganisms 12 (7): 1346. https://doi.org/10.3390/microorganisms12071346
Vaseeharan B, Lin J and Ramasamy P, 2004. Effects of probiotics, antibiotic sensitivity, pathogenicity, and plasmid profiles of *Listonella anguillarum*-like bacteria isolated from *Penaeus monodon* culture systems. Aquaculture 24(1-4): 77-91. https://doi.org/10.1016/j.aquaculture.2004.09.003
Vijaybaskar P and Somasundaram ST, 2008. Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common freshwater pathogen *Aeromonas hydrophila*. Biotechnology 7: 124-128. https://doi.org/10.3923/biotech.2008.124.128
Wuertz S, Schroeder A and Wanka KM, 2021. Probiotics in fish nutrition-long-standing household remedy or native nutraceuticals? Water 13: 1348. https://doi.org/10.3390/w13101348
Yi R, Pan Y, Long X, Tan F and Zhao X, 2020. Enzyme producing activity of probiotics and preparation of compound enzyme. Hindawi Journal of Chemistry 2020. 9140281. https://doi.org/10.1155/2020/9140281
Zapata AA, 2013. Antimicrobial Activities of lactic acid bacteria strains isolated from Nile tilapia intestine (*Oreochromis niloticus*). Journal of Biology and Life Science 4(1): 164-171. http://dx.doi.org/10.13005/bpj/592
Zhang B, Yang H, Cai G, Nie Q and Sun Y, 2024. The interactions between the host immunity and intestinal microorganisms in fish. Applied Microbiology and Biotechnology 108: 30. https://doi.org/10.1007/s00253-023-12934-1
Zokaeifar H, Balcázar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A and Nejat N, 2012. Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology 33(4): 683-689. https://doi.org/10.1016/j.fsi.2012.05.027