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Growth Analysis and Innate Immune Response of Tilapia (*Oreochromis niloticus*) Fed with Synbiotic Feeds in Brackish Water

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ABSTRACT

This study aimed to examine the effects of adding synbiotics to feed on Nile tilapia (*Oreochromis niloticus*) feed conversion efficiency, growth, and innate immune response. Commercial feed was supplemented with 1% prebiotic (banana flour) and the candidate probiotic bacterium *Bacillus subtilis* at doses of zero (control, A); 1x10^{5}CFU/mL (B); 1x10^{7}CFU/mL (C); and 1x10^{9}CFU/mL (D). After eight weeks of feeding the Feed Conversation Ratio (FCR), Weight Gain (WG) and Specific Grow Rate (SGR) were calculated. Biochemical parameters (total erythrocytes, leukocytes, and hematocrit levels) and phagocytic activity were measured from blood samples taken at the end of the experimental period. WG (2.33-3.49g), SGR (1.29-1.61% per day) and FCR (1.05-1.17) did not differ significantly (P>0.05) between treatments. Hematocrit and erythrocyte levels were highest under the control treatment (without probiotics). Hemoglobin (Hb) was highest under treatment B (7.76mg/mL) on day 35; Mean Corpuscular Volume (MCV) (229.35µm^3) and Mean Corpuscular Hemoglobin (MCH) (56.12pg) were highest on day 28, while Mean Corpuscular Hemoglobin Concentration (MCHC) increased over the observation period. The phagocytic index increased under probiotic-enriched feed treatments, indicating that these probiotics could improve leukocyte performance with respect to the phagocytosis of incoming antigens.

Key words: *Bacillus subtilis.*, Growth, Synbiotic, Tilapia

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a freshwater food fish favored by many consumers in Indonesia with good domestic and overseas market prospects (Ibrahem et al. 2010; Huicab-Pech et al. 2017; Abadi et al. 2020). Market demand has been a driving force for the increase in tilapia aquaculture production which, in turn, has increased the volume of feed required by fish farmers (Moura et al. 2016). The price of feed is relatively high due to the high protein content required, and the decreasing availability of quality raw materials (Kok et al. 2020). Therefore, to meet the protein needs of increased fish production, feed efficiency needs to be optimized.

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Feed efficiency can be increased by adding enzyme-producing probiotics to the feed so that it is easier to digest; these enzymes can be effective in increasing growth performance (Afrilasari and Meryandini 2016; Tan et al. 2019; Assan et al. 2022). The activity of the probiotic bacteria contained in the feed improves feed digestibility so that the fish can absorb and convert into growth a higher proportion of the nutrients in the feed (Wedemeyer 1996; Widanarni et al. 2014).

Several studies have found that probiotics can play beneficial roles; for example, they can help to improve growth performance, disease resistance, immune response, gut microbiome composition, and water quality (Dawood et al. 2019; Moustafa et al. 2021; El-Saadony et al. 2021). Probiotics are live microbes that, when administered appropriately, can have a positive effect on the health of the host and improve the balance of the micro-organism community (microbiome) in the digestive tract (Merrifield et al. 2010; Nayak 2010; Jha et al. 2020). However, one weakness limiting the application of probiotics is the varied ability of potentially beneficial probiotic bacteria to survive, colonize, and compete for nutrients in the host gut (Uttamrao 2021; Monica and Jayaraj 2021). One approach to overcome this limitation is to use prebiotics, which contain plant fiber that can serve as food for beneficial bacteria such as those used as probiotics (Khare et al. 2018).

Synbiotic feeds combine probiotic and prebiotic additives to improve feed quality, feed conversion efficiency, fish growth and survival, and the population of lactic acid bacteria in fish guts (Akrami and Arab Arkadeh 2016; Pangaribuan et al. 2017). Pangaribuan et al. (2017) further stated that the addition of synbiotics in Nile tilapia (*O. niloticus*) feed could increase feed efficiency by 55.46%, protein digestibility by 82.41%, specific growth rate by 4.18%, and increase survival rates. The purpose of this study was to evaluate the effects of adding a synbiotic (banana flour as the prebiotic and the bacterium *Bacillus subtilis* as the candidate probiotic) to Nile tilapia (*Oreochromis niloticus*) feed, in terms of feed conversion efficiency, growth, and immune system response.

MATERIALS AND METHODS

Ethical Approval Statements

This *in-vivo* study was carried out in faithful agreement with the ethical guidelines of the ethical principles of the Experimental Health and Animal Welfare Ethics Committee of the Faculty of Medicine, Hasanuddin University. The protocol was approved by the Committee on Research Ethics of the Department of Health and Animals Ethics. Furthermore, efforts were made at all stages to minimize the suffering and pain that could be experienced by the experimental fish.

Experimental Fish

Saline Nile tilapia *Oreochromis niloticus* with an initial weight of ±1g was obtained from the Takalar Brackish Water Aquaculture Centre (N=500) and acclimatized for one week before being released into 500L fiberglass tanks.

Feed Preparation

The commercial feed used was MS Prima Feed PF 1000 with 39-41% protein content (Table 1). The feed was then enriched by spraying with the candidate probiotic bacteria *Bacillus subtilis* at doses of 1 x 10^5CFU/mL, 1 x 10^7CFU/mL, and 1 x 10^9CFU/mL mixed with 1% prebiotic (banana flour). There were three replicates for each treatment and for the control.

Experimental Design

The experimental fish were reared for 30 days in aquaria measuring 50×30×30cm at a density of 20 fish per aquarium (experimental unit). There were four treatments: control (A) with no added probiotic, and synbiotic feed enriched with probiotic at three doses: 1 x 10^5CFU/mL (B), 1 x 10^7CFU/mL (C) and 1 x 10^9CFU/mL (D) with three replicates of each treatment (12 experimental units). The fish were fed 3 times a day with the appropriate feed for each treatment at 08:00, 03:00, and 18:00 WITA, following Hossain et al. (2001). Water quality parameters (temperature, dissolved oxygen, and ammonia concentration) were monitored daily during this research (Table 2). The average water temperature was maintained within the range of 29-32°C, within the appropriate range (24-32°C) according to El-Sayed and Kawanna, 2008). The mean dissolved oxygen level was 7.8±0.5mg/L, within the appropriate range for warmwater aquaculture according to Boyd (1979). Ammonia concentration was 0.008±0.2 mg/L, within the appropriate range according to Gross et al. (1999).

Tilapia Growth Performance

Growth performance was evaluated after an experimental period of eight weeks. The final body weight (BW) and number of fish in each experimental unit were recorded after fasting the fish for 24 hours. Feed Conversation Ratio (FCR), Weight Gain (WG), and Specific Growth Rate (SGR) for each experimental unit were calculated according to the following formulae, with all weights in grams (g):

FCR = (Total weight of experimental fish at the end of the study – Total weight of experimental fish at the beginning of the study) /Amount of feed consumed during the study period (Tacon 1987).

WG (%) = 100 × (Total weight of experimental fish at the end of the study – Total weight of experimental fish at the beginning of the study / Total weight of experimental fish at the beginning of the study

SGR (%BW/Day) = 100 x (Ln (Total weight of experimental fish at the end of the study)–Ln (Total weight of test animals at the beginning of the study))/ rearing period (in days).

Hematological and Immunological Analysis

Hematology parameters were measured by collecting blood samples at the end of the rearing period to determine the total erythrocyte and leukocyte counts (Blaxhall and Daisley 1973), the hematocrit count, and phagocytic activity levels for immunological analysis (Anderson and Siwicki 1993). Phagocytic activity levels were measured on day 15 and day 30. Three fish from each treatment were selected at random and anesthetized in MS-222 solution (50mg/L) before the 0.5mL blood samples were taken. Blood was collected in EDTA anti-coagulant vials. The

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Table 1: Dietary formulation and proximate composition of the commercial feed used as a base for the synbiotic feed trials (%)

Ingredient	% by weight
Fish meal	40
Prawn head flour	8
Soybean meal	15
Coconut flour	5
Wheat gluten	22
Maize gluten	8
Vitamin premix	1.5
Carboxymethyl cellulose (CMC)	0.5
Proximate composition	**% (dry matter basis)**
Crude protein	26.09
Crude fiber	7.48
Crude lipid	4.25
Water content	8.63
Ash content	7.31

blood samples so collected were processed for the study of various hematological parameters following Hesser (1960). Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were measured in the laboratory following the Association of Official Analytical Chemists (AOAC) Official Methods of Analysis℠ (OMA) standards (Orun et al. 2003).

Challenge Test

A challenge test was conducted on the saline tilapia on day 31. The experimental fish were injected intramuscularly with a suspension of the pathogenic bacteria *Aeromonas hydrophila* at a concentration of 1 x 10^6 CFU/mL and a dose of 0.1mL per fish using a sterile syringe following Rahmi et al. (2021). The treated fish in the negative control were transferred to another pond with the same water conditions and injected with 0.1mL Phosphate Buffered Saline (PBS) solution. The saline tilapia was then reared for ten days and given standard commercial feed at a frequency of 3 times/day. Growth performance was observed daily.

Statistical Analysis

Results were tabulated and expressed as mean±SD. Statistical analyses were implemented in SPSS (version 20.0). The data were analyzed using One-Way Analysis of Variance (ANOVA), followed by the post-hoc Tukey test when significant between-treatment differences were found. The significance of between-treatment differences was evaluated at the 95% confidence level (α=0.05).

RESULTS AND DISCUSSION

Tilapia Growth Performance

Growth performance of tilapia (O. *niloticus*) based on weight gain (WG), specific growth rate (SGR) and feed conversion rate (FCR) (Fig. 1) did not differ significantly ($P>0.05$) between treatments. Weight gain (WG) ranged from 2.33-3.49g, SGR ranged from 1.29-1.61% BW/day and FCR ranged from 1.05-1.17.

Probiotics can improve digestion by stimulating the production of enzymes to increase growth performance (Walker and Lim 2011). Synbiotics is a term that refers to nutritional supplements that combine probiotics and prebiotics in a synergistic form (Widanarni et al. 2022). Some studies report that feed enrichment can increase the growth rate of tilapia (O. *niloticus*) (Aly et al. 2008; Samir et al. 2017), including through combined probiotic and prebiotic enrichment formulations. Other studies (He et al. 2013) have also found that the addition of different probiotics to feed did not significantly affect tilapia growth performance. However, despite the lack of statistical significance, treatment B (B. *subtilis* at 10^5 CFU/mL) gave better growth performance than the other treatments in terms of WG (3.49±1.04 g) and SGR (1.38±0.000%). This indicates that synbiotic feed enriched in this way could increase the synergism between probiotics and prebiotics, improving digestive tract function.

Hematological and Immunological Parameters

Mean hematocrit, erythrocyte, and leukocyte counts differed significantly between treatments (Fig. 2). Tilapia hematocrit count was highest in the control treatment without the addition of probiotics (A), followed by the treatment with the highest density of probiotic bacteria (D), and lowest for the treatment with the lowest density of probiotic candidate bacteria (B) (Fig. 2a). The erythrocyte count was also highest in control treatment A, followed by the intermediate probiotic density treatment (C) and lowest in treatment B (Fig. 2b). Conversely, the leukocyte count was highest in treatment B, followed by treatment D and lowest in the control treatment A (Fig. 2c).

The Hb, MCV, MCH and MCHC test results also varied between treatments (Fig. 3). All parameters were the highest on average under treatment B, although the peak levels occurred at different times. Hb concentration was highest in treatment B (7.76mg/mL) on day 35, the MCV (229.35µm^3) and MCH (56.12pg) peaked on day 28, and the MCHC increased with observation time.

Mean phagocytic activity (Fig. 3) varied between treatments after 15 days of rearing (A 75.25%, B 89.75%, C 84.2%, and D 81.75%) and 30 days of rearing (A 72.58%, B 99.5%, C 87.5% and D 87.5%). However, within-treatment variation was also quite high, so the differences were not significant. with quite variable values during sampling.

Blood hematology parameters are one group of physiological biomarkers that can be used to evaluate fish health (Dossou et al. 2019). Parameters related to red blood cell volume measurement are hematocrit and erythrocyte counts. The hematocrit count represents the percentage volume of red blood cells in the blood. Hematocrit typically accounts for 20-30% of red blood cells in teleost fish (Boyd 1979) and a decrease in blood hematocrit is an indicator of

Table 2: Nile tilapia rearing media water quality parameter ranges during the study period

Parameter	Range by Treatment				Tolerance Range	References
	A	B	C	D		
Temperature (°C)	29-32	29-32	29-32	29-31	24-32	El-Sayed and Kawanna (2008)
DO (ppm)	6.4-7.8	6.7-7.9	6.7-7.9	6.7-7.8	6.1-9.5	Boyd et al. (1979)
Ammonia (ppm)	0.008-0.012	0.008-0.013	0.009-0.013	0.009-0.013	<0.02	Gross et al. (1999)

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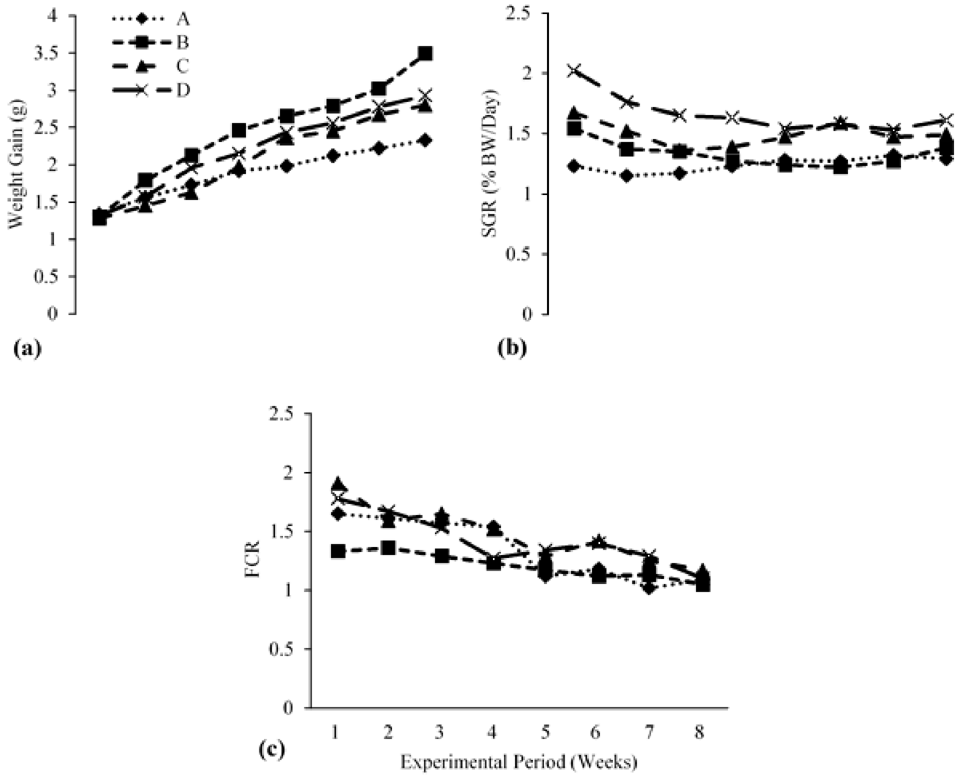
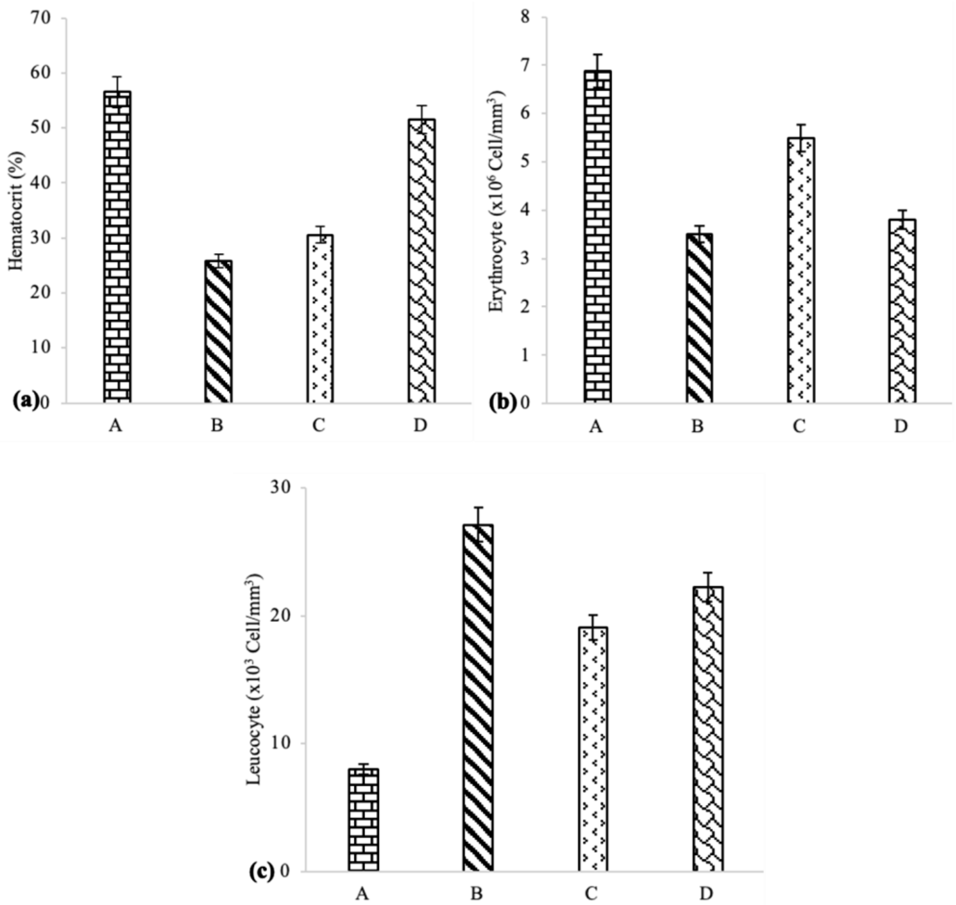


5 **Fig. 1:** Growth performance of O. *niloticus* over a rearing period of 8 weeks (n=3*): (a) Weight gain (WG); (b) Specific growth rate (SGR); and (c) Feed conversion ratio (FCR) under four treatments. A=control; B=1 x 10^5CFU/mL probiotic; C=1 x 10^7CFU/mL probiotic; and D=1 x 10^9CFU/mL probiotic. *The n=3 refers to the number of replicate units, each with 6 fish and each with a mean value. This mean is therefore the sum of mean of 3x6=18 fish.



5 **Fig. 2:** Tilapia hematocrit (a), erythrocyte (b) and leukocyte (c) counts (mean±SD) after eight weeks of rearing under four probiotic enrichment treatments: control (A); 1 x 10^5CFU/mL (B); 1 x 10^7CFU/mL (C); and 1 x 10^9CFU/mL (D) (n=3*). *The n=3 refers to the number of replicate units, each with 6 fish and each with a mean value. This mean is therefore the sum of mean of 3x6=18 fish.

31 stress in fish (Hoseini et al. 2018). Under normal conditions, erythrocytes comprise almost half of the blood volume, and the normal erythrocyte count range in tilapia is 1.05-3.5×10^6 cells/mm^3 (Putranto et al. 2019). Research by Reda and Selim (2015) showed an increase in hemoglobin and hematocrits in tilapia (O. *niloticus*) fed *Bacillus amyloliquefaciens*. Based on Fig. 2, the tilapia in treatments A and C had high hematocrit and erythrocyte levels. In contrast, the tilapia in treatment B had normal hematocrit and erythrocyte levels.

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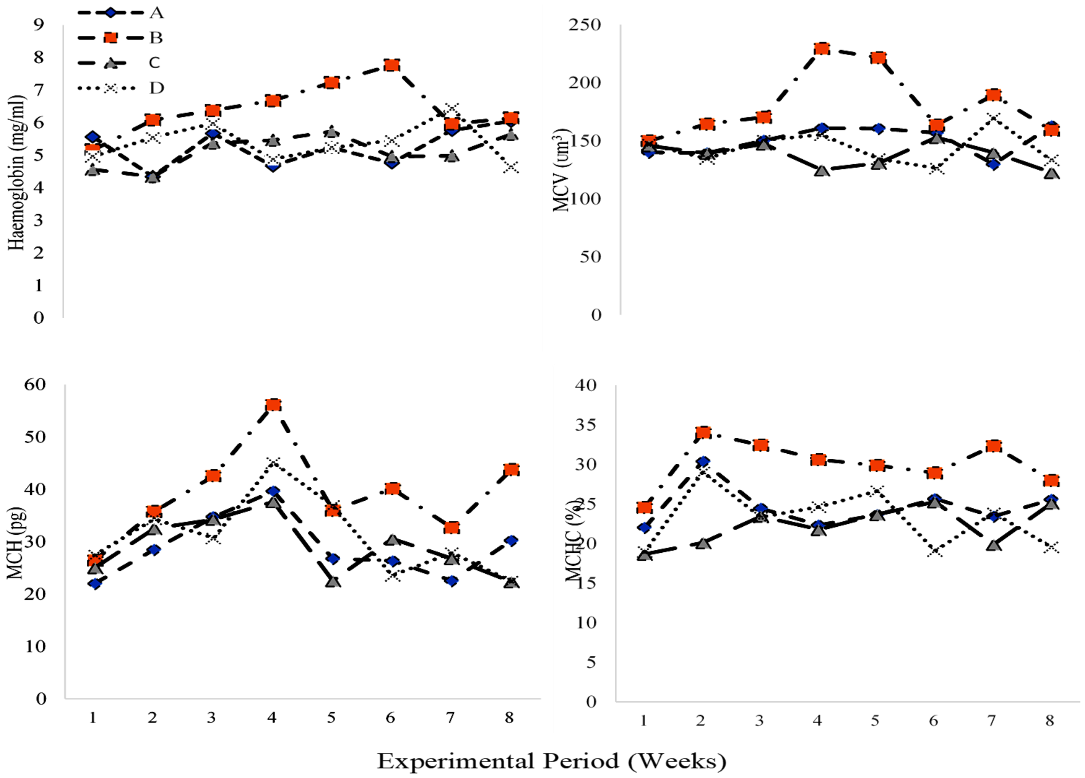

Fig. 3: Mean value of blood parameters of O. *niloticus* (n=3) during rearing under four probiotic enrichment treatments (control (A); 1 x 10^5CFU/mL (B); 1 x 10^7CFU/mL (C); and 1 x 10^9CFU/mL (D)): (a) Hemoglobin; (b) MCV=mean corpuscular volume; (c) MCH=mean corpuscular hemoglobin; (d) MCHC=mean corpuscular hemoglobin concentration.

Kord et al. (2021) found that several blood parameters (red blood cells, white blood cells, hemoglobin, and hematocrits) increased in tilapia fed a probiotic-fortified diet. Decreased hematocrit levels in the blood can indicate anemia (Putranto et al. 2019). In cases such as microcytic anemia, the number and size of red blood cells are reduced, so the hematocrit level is also low (Ahmed et al. 2022). If exposed to infection, anemia can be caused by a decrease in appetite due to these pathogenic infections, leading to a decrease in hematocrit levels, which in turn leads to anemia (Docan et al. 2018). Conversely, sharp increases in hematocrit levels could be due to stress experienced by the fish (Wedemeyer and Yasutake 1977). These mechanisms could explain the high hematocrit levels in the control treatment, without probiotic enrichment.

The hematocrit test measures the proportion of red blood cells in the blood. Hematocrit and erythrocyte levels in treatment A (without additional probiotics) increased during the study's observation period. Meanwhile, treatment B (synbiotic feed enhanced with probiotics at a dose of 1x10^7 CFU/mL) produced the most significant leukocyte count. Mulyadi et al. (2021) suggested that erythrocyte values can fluctuate due to the inhibition of erythropoiesis from bacterial infection injected into tilapia (O. *niloticus*). Furthermore, an increase in hematocrit and erythrocyte counts can indicate that fish are experiencing stress, e.g., due to the salinity of the rearing medium (Paul et al. 2022), while an increase in leukocyte counts in the fish's blood after being fed synbiotic feed is most likely an indication that the immune system has successfully mounted a cellular response (Rohani et al. 2022).

A decrease in the total number of erythrocytes in the body means there is a decrease in hematocrits, which can indicate the occurrence of anemia (Wedemeyer and Yasutake 1977; Irianto and Austin 2002; Kumar et al. 2008). A decrease in erythrocytes can also occur due to phagocytosis of bacteria that infect fish, a process that requires oxygen (Lestari et al. 2018). The bacteria present in the fish body will be phagocytized after the bacterial particles are recognized and digested by phagocytic cells that require oxygen, causing a decrease in the number of erythrocytes (Rodrigues et al. 2020; Prasetyo et al. 2021).

Leukocytes are blood cells that play a role in the immune system. Leukocytes help rid the body of foreign substances, including invading pathogens through the immune system and other responses (Moyle and Cech 2004). The addition of prebiotics in feed can increase the leukocyte count in tilapia (Hartika et al. 2014: Marina et al. 2015). The observed leukocyte counts in treatment B (27.120±1.465) fall within the normal standard range for tilapia, which is 20,000 cells/mm^3-150,000 cells/mm^3 according to Widanarni et al. (2014). Fish condition and health parameters are factors that can affect the leukocyte count. The total number of leukocytes increased during the addition of prebiotics in tilapia feed compared to beforehand, indicating that the prebiotics played a significant role in increasing tilapia resistance to disease and infection. According to Sirimanapong et al. (2018), dangerous levels of pathogen attack are indicated by leukocyte counts reaching 40-50% of the total cell count. Butprom et al. (2013) found that the administration of an immunostimulant supplemented with bacteria in the feed could increase the phagocytic activity of leukocytes after 45 days in catfish.

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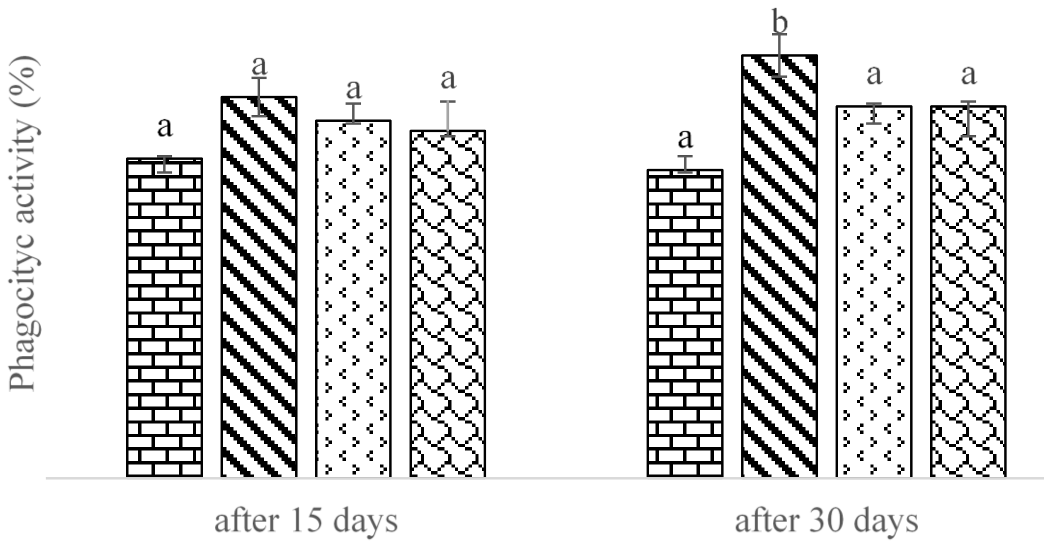

Fig. 4: Phagocytic activity in tilapia (mean ± SD, n=3) after 15 days and 30 days of rearing under four probiotic enrichment treatments: control (A); 1 x 10^5CFU/mL (B); 1 x 10^7CFU/mL (C); and 1 x 10^9CFU/mL (D). (Note: Different superscript letters on the same picture showed significant differences (P<0.05) between treatments).

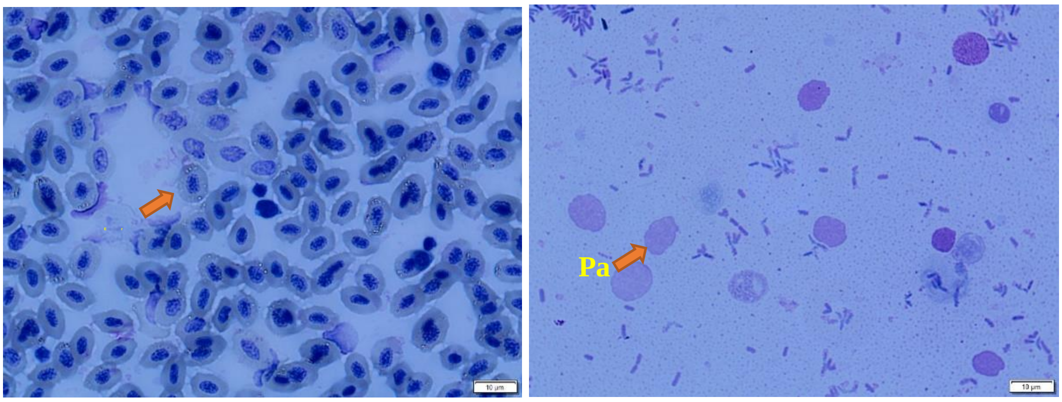

Fig. 5: Tilapia (*Oreochromis niloticus*) blood slides (Hematoxylin and Eosin-stained sections, 100x magnification, 10µm scale bars): arrows indicate Erythrocytes (Er) and Phagocytic activity (Pa).

The results of this study further revealed that specific blood parameters such as Hb, MCV, MCH, and MCHC were higher in treatment B (1x10^5 CFU/mL) (Fig. 3), Thus, this treatment could improve the health of fish reared under aquaculture conditions and thereby improve fish production. As in other cold-blooded vertebrates, seasonal and other environmental effects can affect tilapia physiology and blood biochemistry (Ranzani-Paiva et al. 2004; Xia et al. 2020). Hematological studies have generally been used as an effective and sensitive index for monitoring physiological changes in fish, as they can provide valuable information about the external environmental response to the internal physiology of fish (Kumar et al. 2017).

The increase in leukocytes is thought to be caused by the invasion of bacteria, which triggers the defense system to produce leukocytes which can circulate in the blood and reach the infected tissues. The leukocytes produced are used to rid the body of pathogens (Lestari et al. 2018). Furthermore, Elkamel and Mosaad (2012) showed that probiotics could increase leukocyte abundance and stimulate lymphoid tissue to trigger innate immune system response.

Phagocytosis is one of the innate immune response mechanisms which defend fish against attack by pathogenic microorganisms (Khaled et al. 2015; Biller and Takahashi 2018). The phagocytic index varied during the research period and differed between the sampling on day 15 and day 30. Fig. 3 shows that treatment with the addition of prebiotic at a dose of 10^5 CFU/mL in tilapia feed could increase immune system response. The phagocytic process is an immune response mechanism that is part of the body's self-defense against infection from pathogenic microorganisms (Tizard, 1982; Kumar et al. 2008; Lestari et al. 2018; Moustafa et al. 2021). The increased phagocytic index shows that the prebiotic increased phagocytosis of incoming antigens by leukocytes in the fish blood (Kurniawan et al. 2019). The phagocytic index reflects the level of aggressiveness of leukocytes in destroying antigens that enter the body (Nicholson 2016).

This study shows that the administration of probiotics through feed can increase the phagocytic index of treated fish compared to the control group which did not receive probiotic treatment, as also reported by other studies on probiotic feed additives (Telli et al. 2014; Khaled et al. 2015). The increase in the phagocytic index indicates that the addition of probiotics was able to improve leukocyte performance with respect to the phagocytosis of incoming antigens. This is in line with the findings of Elkamel and

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Mosaad (2012) that feeding probiotics to aquatic animals can significantly increase phagocyte activity and the phagocytic index.

Based on blood parameters, synbiotic feed enrichment with B. *subtilis* at a dose of 1x10^5 CFU/mL gave the best results in saline tilapia infected with A. *hydrophila* bacteria. Under this treatment, the infected fish had erythrocyte, hematocrit, and leukocyte counts and phagocytic activity levels within the normal range. This is consonant with the findings of Cavalcante et al. (2020) that the addition of probiotics and prebiotics to feed can reduce the mortality of tilapia infected with pathogens by boosting innate immunity and increasing leukocyte counts. The results of this research show that the addition of the synbiotic to saline tilapia feed can improve growth performance, non-specific immune system function, and disease resistance.

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Conclusion

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Based on the study's results, feeding synbiotics with B. *subtilis* at 10^5 CFU/mL can provide better growth performance (WG and SGR), although not significantly different between treatments. Hb, MCV, MCH, and MCHC were found to be higher in treatment synbiotic feed enriched with probiotics at doses 1 x 10^5 CFU/mL. This shows that synbiotic feed enriched in this way can increase the synergy between probiotics and prebiotics to improve digestive tract function. Thus, the performance of fish health conditions in fish farming and production can be improved.

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REFERENCES

Ahmed I, Zakiya A and Fazio F, 2022. Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: a review. Frontiers in Environmental Science 10: 919204. https://doi.org/10.3389/fenvs.2022.919204

Anderson DP and Siwicki AK, 1993. Basic hematology and serology for fish health programs. Paper presented in second symposium on disease in Asian Aquaculture "Aquatic Animal Health and Environment". Phuket Thailand 1: 10–17.

Abadi AS, Hismayasari IB, Supriatna I, Yani A and Sayuti M, 2020. The mass death of Nile tilapia (*Oreochromis niloticus*) in Sorong District, West Papua, Indonesia. AACL Bioflux 13: 1906-1916.

Afrilasari W and Meryandini A, 2016. Effect of probiotic *Bacillus megaterium* PTB 1.4 on the population of intestinal microflora, digestive enzyme activity and the growth of catfish (*Clarias* sp.). HAYATI Journal of Biosciences 23: 168-172. https://doi.org/10.1016/j.hjb.2016.12.005

Akrami R and Arab Arkadeh M, 2016. Effect of dietary synbiotics on growth, immune response and body composition of Caspian roach (*Rutilus rutilus*). Iranian Journal of Fisheries Sciences 15: 170-182.

Aly SM, Ahmed YAG, Ghareeb AAA and Mohamed MF, 2008. Studies on *Bacillus subtilis* and *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. Fish and Shellfish Immunology 25: 128-136. https://doi:10.1016/j.fsi.2008.03.013

Assan D, Kuebutornye FKA, Hlordzi V, Chen H, Mraz J, Mustapha UF and Abarike ED, 2022. Effects of probiotics on digestive enzymes of fish (finfish and shellfish); status and prospects: a mini review. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 257: 110653. https://doi.org/10.1016/j.cbpb.2021.110653

Biller JD and Takahashi LS, 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. Anais da Academia Brasileira de Ciências 90: 3403-3414. https://doi.org/10.1590/0001-3765201820170730

Blaxhall PC and Daisley KW, 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology 5: 577–581. https://doi.org/10.1111/j.1095–8649.1973.tb04510.x

Butprom S, Phumkhachorn P and Rattanachaikunsopon P, 2013. Effect of *Lactobacillus plantarum* C014 on innate immune response and disease resistance against *Aeromonas hydrophila* in hybrid catfish. The Scientific World Journal 2013: 392523. http://dx.doi.org/10.1155/2013/392523

Boyd EC, 1979. Water quality in warmwater fishponds. Auburn University, Auburn, USA, pp: 359.

Cavalcante RB, Telli GS, Tachibana L, Dias DC, Oshiro E, Natori MM, Silva WF and Ranzani-Paiva MJ, 2020. Probiotics, prebiotics and synbiotics for Nile tilapia: Growth performance and protection against *Aeromonas hydrophila* infection. Journal Aquaculture Report 17: 1-8. https://doi.org/10.1016/j.aqrep.2020.100343

Dawood MA, Koshio S, Abdel-Daim MM and Van Doan H, 2019. Probiotic application for sustainable aquaculture. Reviews in Aquaculture 11: 907-924. https://doi.org/10.1111/raq.12272

Dossou S, Kashio S, Ishikawa M, Yokoyama S, El-Basuini MF, Zaineldin AI, Mzengereza, K, Moss A and Dawood MAO, 2019. Effect of replacing fishmeal with fermented and non-fermented rapeseed meal on the growth, immune and antioxidant responses of red sea bream *Pagrus major*. Aquaculture Nutrition 25: 508-517. https://doi.org/10.1111/anu.12876

Docan A, Grecu I and Dediu L, 2018. Use of hematological parameters as assessment tools in fish health status. Journal of Agroalimentary Processes and Technologies 24: 317-324.

El-Saadony MT, Alagawany M, Patra AK, Kar I, Tiwari R, Dawood MA, Dhama K and Abdel-Latif HM, 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

Hesser EF, 1960. Methods for routine fish hematology. The Progressive Fish-Culturist 22: 164-171. https://doi.org/10.1577/15488659(1960)22[164:MFRFH]2.0.CO;2

El-Sayed AF and Kawanna M, 2008. Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquaculture Research 39(6): 670. https://doi:10.1111/j.1365-2109.2008.01915.x.

Elkamel AA and Mosaad GM, 2012. Immunomodulation of Nile Tilapia, *Oreochromis niloticus*, by *Nigella sativa* and *Bacillus subtilis*. Journal of Aquaculture Research and Development 3: 1–4. https://doi:10.4172/2155–9546.1000147

Gross A, Boyd CE and Wood CW, 1999. Ammonia volatilization from freshwater fish ponds. Journal of Environmental Quality 28: 93–797. https://doi:10.2134/jeq1999.00472425002800030009x

Hartika R, Mustahal M and Putra AN, 2014. Gambaran darah ikan nila (*Oreochromis niloticus*) dengan penambahan dosis

Int J Vet Sci, 2024, 13(3): 291-299.

prebiotik yang berbeda dalam pakan. Jurnal Perikanan dan Kelautan 4: 259–267. https://doi.org/10.33512/jpk.v4i4.174

Huicab-Pech ZG, Castaneda-Chavez MR and Lango-Reynoso F, 2017. Pathogenic bacteria in *Oreochromis niloticus* var. Stirling tilapia culture. Fisheries and Aquaculture Journal 8: 197. https://doi:10.4172/2150-3508.1000197

He S, Zhang Y, Xu L, Yang Y, Marubashi T, Zhou Z and Yao B, 2013. Effects of dietary Bacillus subtilis C-3102 on the production, intestinal cytokine expression and autochthonous bacteria of hybrid tilapia *Oreochromis niloticus* ♀×*Oreochromis aureus* ♂. Aquaculture 412–413: 125-130. https://doi.org/10.1016/j.aquaculture.2013.06.028

Hossain M, Haylor GS and Beveridge MCM, 2001. Effect of feeding time and frequency on the growth and feed utilization of African catfish *Clarias gariepinus* (Burchell 1822) Fingerlings. Aquaculture Research 32: 999–1004. https://doi:10.1046/j.1365–2109.2001.00635.x

Hoseini SM, Hoseinifar SH and Doan HV, 2018. Effect of dietary eucalyptol on stress markers, enzyme activities and immune indicators in serum and haematological characteristics of common carp (*Cyprinus carpio*) exposed to toxic concentration of ambient copper. Aquaculture Research 49: 3045-3054. https://doi.org/10.1111/are.13765

Ibrahem MD, Fathi M, Mesalhy S and Abd El-Aty AM, 2010. Effect of dietary supplementation of inulin and vitamin C on the growth, hematology, innate immunity and resistance of Nile tilapia (*Oreochromis niloticus).* Fish and Shellfish Immunology 29: 241-246. https://doi.org/10.1016/j.fsi.2010.03.004

Irianto A and Austin B, 2002. Probiotics in aquaculture. Journal of Fish Disease 25: 633-642. https://doi.org/10.1046/j.1365-2761.2002.00422.x

Jha R, Das R, Oak S and Mishra P, 2020. Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. Animals 10: 1863. https://doi.org/10.3390/ani10101863

Khaled M, Selim, Rasha M and Reda, 2015. Improvement of immunity and disease resistance in the Nile tilapia, *Oreochromis niloticus*, by dietary supplementation with *Bacillus amyloliquefaciens*. Fish and Shellfish Immunology 44: 496-503. https://doi.org/10.1016/j.fsi.2015.03.004

Khare A, Thorat G, Bhimte A and Yadav V, 2018. Mechanism of action of prebiotic and probiotic. Journal of Entomology and Zoology Studies 6: 51-53

Kord MI, Tarek Mohamed Srour TM, Omar EA, Farag AA, Nour AAM and Khali HS, 2021. The Immunostimulatory effects of commercial feed additives on growth performance, non-specific immune response, antioxidants assay, and intestinal morphometry of Nile tilapia, *Oreochromis niloticus*. Frontiers in Physiology 12: 627449. https://doi.org/10.3389/fphys.2021.627499

Kurniawan A, Suminto S and Haditomo A, 2019. The effect of probiotic candidate bacteria *Bacillus methylothropicusin* commercial feed on blood profile and growth performance tilapia (*Oreochromis niloticus*) tested by *Aeromonas hydrophila*. Indonesian Journal of Tropical Aquaculture 3: 82–92. https://doi.org/10.14710/sat.v3i1.3956

Kumar R, Mukherjee SC, Ranjan R and Nayak SK, 2008. Enhanced innate immune parameters in *Labeo rohita* (Ham.) following oral administration of *Bacillus subtilis*. Fish and Shellfish Immunology 24:168-172. https://doi:10.1016/j.fsi.2007.10.008

Kumar SP, Sharma BK, Sharma SK and Upadhyay B, 2017. Comparative haematology of pre and post spawning common carp (*Cyprinus carpio*). Journal of Entomology and Zoology Studies 5: 1793-1798.

Kok B, Malcorps W, Tlusty MF, Eltholth MM, Auchterlonie NA, Little DC, Harmsen R, Newton RW and Davies SJ, 2020. Fish as feed: Using economic allocation to quantify the Fish In: Fish Out ratio of major fed aquaculture species. Aquaculture 528: 735474. https://doi.org/10.1016/j.aquaculture.2020.735474

Lestari S, Rahmawati FF and Jumadi R, 2018. Pengaruh penambahan serbuk daun tanaman kayu manis (*Cinnamomum burmannii*) pada pakan terhadap profil darah (kadar hematokrit, kadar hemoglobin, total leukosit dan total eritrosit) ikan nila (*Oreochromis niloticus*) yang diinfeksi *Streptococcus agalactiae*. Jurnal Perikanan Pantura 1: 24–31. http://dx.doi.org/10.30587/jpp.v1i1.289

Marina Keiko P, Iwashita, Ivan B, Nakandakare, Jeffery S, Terhune, Wood T, Maria José T and Ranzani P, 2015. Dietary supplementation with *Bacillus subtilis*, *Saccharomyces cerevisiae* and *Aspergillus oryzae* enhance immunity and disease resistance against *Aeromonas hydrophila* and *Streptococcus iniae* infection in juvenile tilapia *Oreochromis niloticus*. Fish and Shellfish Immunology 43: 60-66, https://doi.org/10.1016/j.fsi.2014.12.008

Mulyadi A, Widarni, Yuhana M and Wahjuningrum D, 2021. Growth performance, immune response, and resistance of Nile tilapia fed paraprobiotic *Bacillus* sp. NP5 against *Streptococcus agalactiae* infection. Jurnal Akuakultur Indonesia 20: 34–46. https://doi:10.19027/jai.20.1.34-46

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

Monica KS and Jayara EG, 2021. Review on probiotics as a functional feed additive in aquaculture. International Journal of Fisheries and Aquatic Sciences 9: 201-207. https://doi.org/10.22271/fish.2021.v9.i4c.2528

Moyle PB and Jr J Cech, 2004. Fishes: An Introduction to Ichthyology. Prentice Hall, USA, pp: 597.

Moura RST, Valenti WC and Henry-Silva GG, 2016. Sustainability of Nile tilapia net-cage culture in a reservoir in a semi-arid region. Ecological indicators 66: 574-582. https://doi.org/10.1016/j.ecolind.2016.01.052

Moustafa EM, Farrag FA, Dawood MA, Shahin K, Hamza A, Decamp O, Mohamed R, Elsabagh M, Eltholth M and Omar AA, 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: 2205-2219. https://doi.org/10.1111/are.15073

Nayak SK, 2010. Probiotics and Immunity: A Fish Perspective. Journal Fish Immunology 29: 2–14. http://doi:10.1016/j.fsi.2010.02.017

Nicholson LB, 2016. The immune system. Essays in Biochemistry 60: 275-301. https://doi.org/10.1042/EBC20160017

Orun, Doruca M and Yazlak H, 2003. Hematological parameters of three Cyprinid fish species from Karakaya Dam Lake, Turkey. Online Journal of Biological Science 3: 320-328. https://doi.org/10.3923/JBS.2003.320.328

Pangaribuan E, Sasanti AD and Amin M, 2017. Efisiensi pakan, pertumbuhan, kelangsungan hidup dan respon imun ikan patin (*Pangasius* sp.) yang diberi pakan bersinbiotik. Jurnal Akuakultur 5: 140–154. https://doi.org/10.36706/jari.v5i2.7139

Paul M, Sardar P, Sahu NP, Deo AD, Varghese T, Shamna N, Jana P and Krishna G, 2022. Effect of dietary protein level on growth and metabolism of GIFT juveniles reared in inland ground saline water of medium salinity. Journal of Applied Aquaculture 2022: 2054672. https://doi.org/10.1080/10454438.2022.2054672

Putranto, Wahyu D, Syaputra D and Prasetiyono E, 2019. Gambaran darah ikan nila (*Oreochromis niloticus*) yang diberi pakan terfortifikasi ekstrak cair daun salam. Jurnal

Aquatropica Asia 4: 22–28. https://doi.org/10.33019/aquatropica.v4i2.2222

Prasetyo D, Zubaidah A, Handajani H and Hudin E, 2021. The effectiveness of cellulolytic bacteria on feeds towards the immune response of Tilapia. Indonesian Journal of Tropical Aquatic 4: 51-61. https://doi.org/10.22219/ijota.v4i2.20360

Rahmi R, Relatami ANR, Akmal A, Firman SW, Tampangallo BR, Chadijah A and Ardiayana, D, 2021. The growth performance of tilapia (*Oreochromis niloticus*) seeds fed with different quantities of a synbiont feed. Jurnal Perikanan Universitas Gadjah Mada 23: 113-118. https://doi.org/10.22146/jfs.68510

Ranzani-Paiva MJT, Ishikawa CM, Eiras ACD and Silveira VRD, 2004. Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757). Brazilian Archives of Biology and Technology 47: 945-953. https://doi.org/10.1590/S1516-89132004000600014

Reda RM and Selim KM, 2015. Evaluation of *Bacillus amyloliquefaciens* on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, *Oreochromis niloticus*. Aquaculture International 23: 203–217. http://doi.org/10.1007/s10499-014-9809-z

Rodrigues MV, Zanuzzo FS, Koch JFA, de Oliveira CAF, Sima P and V, 2020. Development of fish immunity and the role of β-Glucan in immune responses. Molecules 25: 5378. https://doi.org/10.3390/molecules25225378

Rohani MF, Islam SM, Hossain MK, Ferdous Z, Siddik MA, Nuruzzaman M, Padeniya U, Brown C and Shahjahan M, 2022. Probiotics, prebiotics and synbiotics improved the functionality of aquafeed: Upgrading growth, reproduction, immunity and disease resistance in fish. Fish and Shellfish Immunology 120: 569-589. https://doi.org/10.1016/j.fsi.2021.12.037

Samir T, El-Naby ASA, Khattaby AA and Awad SMM, 2017. Improvement of growth rate, feed utilization, body composition and immune response of Nile tilapia (*Oreochromis niloticus*) by supplementation of nucleotide in diets. 1st International Conference. Central Laboratory for Aquaculture Research in Cooperation with WorldFish, Cairo, Egypt, pp: 62-88.

Sirimanapong W, Thompson KD, Shinn AP and Adams A, 2018. *Streptococcus agalactiae* infection kills red tilapia with chronic *Francisella noatunensis* infection more rapidly than the fish without the infection. Fish and Shellfish Immunology 81: 221-232. https://doi.org/10.1016/j.fsi.2018.07.022

Tan HY, Chen SW and Hu SY, 2019. Improvements in the growth performance, immunity, disease resistance, and gut microbiota by the probiotic *Rummeliibacillus stabekisii* in Nile tilapia (*Oreochromis niloticus*). Fish and Shellfish Immunology 92: 265-275. https://doi.org/10.1016/j.fsi.2019.06.027

Tizard IR, 1982. Pengantar Imunologi Veteriner [An Introduction to Veterinary Immunology]. 2nd Edition. Transl.: Partodiredjo, M. Surabaya. Airlangga University Press, pp: 498.

Telli GS, Ranzani-Paiva MJT, de Carla Dias D, Sussel FR, Ishikawa CM and Tachibana L, 2014. Dietary administration of *Bacillus subtilis* on hematology and non-specific immunity of Nile tilapia *Oreochromis niloticus* raised at different stocking densities. Fish and Shellfish Immunology 39: 305-311. https://doi.org/10.1016/j.fsi.2014.05.025

Uttamrao DJ, 2021. Modern techniques in aquaculture: Probiotics in aquaculture. International Journal for Innovative Research in Multidisciplinary Field, Conference Special Issue 22: 145-147.

Walker TL and Lim C, 2011. Use of probiotics in diets of tilapia. Journal of Aquaculture Research and Development S1: 014. http://dx.doi.org/10.4172/2155-9546.S1-014

Widanarni WFA and Yuhana M, 2014. Aplikasi probiotik, prebiotik dan sinbiotik melalui pakan untuk meningkatkan respon imun dan kelangsungan hidup ikan nila *Oreochromis niloticus* yang diinfeksi Streptococcus agalactiae. Jurnal Sains Terapan: Wahana Informasi dan Alih Teknologi Pertanian 4: 15–26. https://doi.org/10.29244/jstsv.4.1.15-26

Widanarni S, Anggraeni AA, Maharani RBA, Mulyadin A, and Yuhana M, 2022. Different dosage applications of *Bacillus* sp. NP5 para-probiotic on the growth performance and resistance of Nile tilapia against *Streptococcus agalactiae* infection. Jurnal Akuakultur Indonesia 21: 186-197. https://doi.org/10.19027/jai.21.2.186-197

Wedemeyer GA and Yasutke, 1977. Clinical methods for the assessment on the effect of environmental stress on fish health. Fish and Wildlife Service Technical Paper TP89, US Department of the Interior, pp: 1-17.

Wedemeyer GA, 1996. Physiology of fish in intensive culture systems. Springer Science and Business Media 227 pp. https://doi.org/10.1007/978-1-4615-6011-1

Xia Y, Wang M, Gao F, Lu M and Chen G, 2020. Effects of dietary probiotic supplementation on the growth, gut health, and disease resistance of juvenile Nile tilapia (*Oreochromis niloticus*). Animal Nutrition 6: 69-79. https://doi.org/10.1016/j.aninu.2019.07.002