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Lactobacillus-Fermented *Oecophylla Smaragdina* Larvae as a Feed Supplement: Effects on Water Quality, Growth, and Health of Tilapia During the Nursery Phase

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ABSTRACT

The success of the Nile tilapia (*Oreochromis niloticus*) aquaculture depends on providing nutritionally balanced feed that adequately meets the species' growth requirements, particularly during the nursery phase. Inefficient feed absorption during this period increases production costs, primarily due to higher feed and maintenance expenses. One promising approach to address this challenge involves using alternative feed sources, such as weaver ant (*Oecophylla smaragdina*) larvae fermented with *Lactobacillus* bacteria. This study evaluated the optimal dosage of pellet feed fortified with fermented *O. smaragdina* larvae by analyzing hematological parameters, gut morphology, growth performance, and nitrogen levels in the *O. niloticus* rearing environment. A completely randomized design was employed, with four dosages tested, each replicated three times. The fermentation dosages of *O. smaragdina* larvae used were 0 (d_0), 10 (d_{10}), 15 (d_{15}), and 20% (d_{20}), with protein contents of 28, 30.95, 31.02, and 32.25%, respectively. The d_{15} dosage was the most effective, promoting weight gain, length growth, survival rate, and feed conversion ratio while positively affecting hematological parameters, nutrient absorption, and water quality. Although the d_{20} dosage resulted in the highest *Lactobacillus* levels, it produced toxic total ammonia nitrogen and nitrite levels, inducing physiological stress and reduced growth performance. Proper water quality management is essential to mitigate harmful conditions when using dosages higher than 15%.

INTRODUCTION

Feed is the most critical component in the farming of the Nile tilapia (*Oreochromis niloticus*) **(Nguyen *et al.*, 2021)**. It serves as the fish's primary source of nutrients and energy, essential for their survival and growth **(Zhang *et al.*, 2020)**. Moreover, the growth rate of *O. niloticus* is significantly influenced by the nutritional quality of the feed. Ineffective and inefficient feed absorption during the nursery phase can lead to

increased production costs, particularly during the rearing period, which, in turn, raises overall operational expenses, including those associated with feed and maintenance **(Ayisi *et al.*, 2017)**. Additionally, providing high-protein commercial feed in Indonesia and other developing countries is prohibitively expensive **(Pahlow *et al.*, 2015)**. One approach to mitigating these challenges is through feed fortification.

A key strategy for improving feed utilization efficiency involves incorporating probiotics **(Liao *et al.*, 2017)**. Probiotics are microorganisms capable of modifying the composition of bacterial populations in the digestive tract, as well as water and sediment **(Średnicka *et al.*, 2017)**. Probiotics containing *Lactobacillus* sp. produced through fermentation have been added to commercial feeds, enhancing fish growth rates **(Flefil *et al.*, 2022)**. Likewise, the fermentation of feed using *Lactobacillus* sp. in *O. niloticus* has been shown to improve digestion and growth **(Islam *et al.*, 2021)**. This is because *Lactobacillus* sp., a lactic acid bacterium, possesses heterofermentative capabilities that allow it to metabolize various sugars and carbohydrates, producing significant quantities of lactic acid **(Ngouénam *et al.*, 2021)**.

Fermentation by *Lactobacillus* sp. induces chemical alterations in organic materials, reducing crude fiber content while increasing crude protein levels **(Dev *et al.*, 2024)**. Among the organic materials with considerable potential is the weaver ant (*Oecophylla smaragdina*) **(Exélis *et al.*, 2024)**. The larvae of this species contain 493 kcal/g of energy, 24.1g of protein, 42.2g of fat, 4.3g of carbohydrates, 230mg of phosphorus, 10.4mg of iron, 710 IU of vitamin A, 0.22mg of vitamin B1, 1.13mg of vitamin B2, and 5.7mg of niacin per 100 grams **(Alagappan *et al.*, 2021)**. However, there is limited research on the properties of fermented *O. smaragdina* larvae, particularly in their application as a supplement in fish feed. *O. smaragdina* inhabits forested areas along riverbanks, eucalyptus trees, and shrubland, nesting in low-vegetation microhabitats **(Correa *et al.*, 2023)**. The species is distributed across various regions, including Sri Lanka, Pakistan, Bangladesh, Nepal, Bhutan, southern China, Taiwan, Southeast Asia, and other tropical countries **(Sabri *et al.*, 2023)**.

This study aimed to evaluate the supplementation of pellet feed with *Oecophylla smaragdina* larvae fermented using *Lactobacillus* sp. and its effects on the growth and health of *Oreochromis niloticus* during the nursery phase, as illustrated in Fig. (1). Key parameters observed included hematology, gut histology, the abundance of *Lactobacillus* sp. in the gut, water quality, and fish growth. Hematological indicators—such as leukocyte count, hematocrit, hemoglobin, and erythrocyte levels—were used to assess the health and physiological condition of the fish, as well as their response to environmental stressors. Histological analysis of the *O. niloticus* gut was conducted to examine how nutrition influences intestinal tissue structure. The study also measured nitrogen levels (ammonia, nitrite, and nitrate) in the rearing medium to assess water quality, as these compounds, influenced by protein excretion, directly affect fish health.

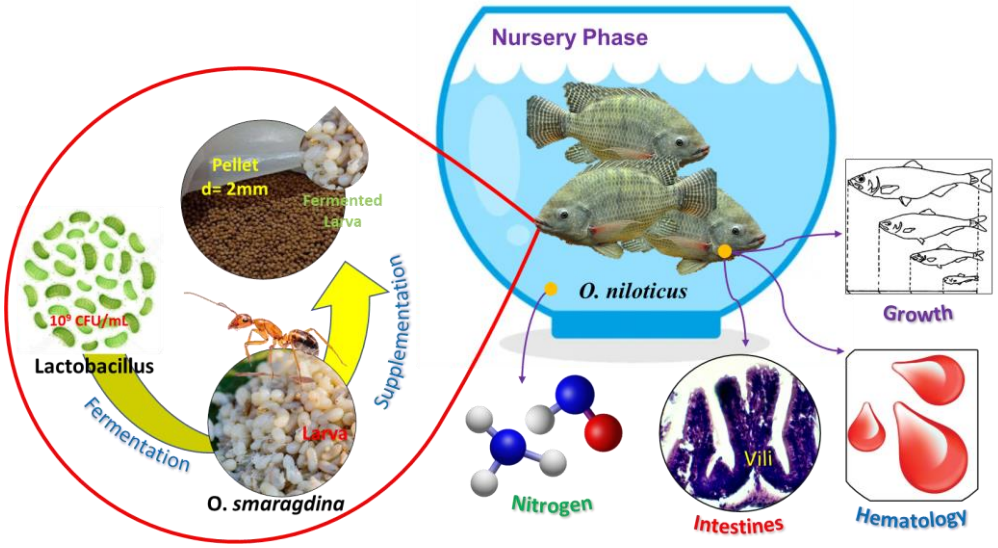

Fig. 1. Supplementation concept *Lactobacillus*-fermented *Oecophylla smaragdina* larvae as a feed supplement for the Nile tilapia (*Oreochromis niloticus*) in the nursery phase

MATERIALS AND METHODS

Preparation of test diets

This study employed a completely randomized design (CRD) with four treatment groups, using 2mm pellets (Ruby HG-2, De Heus Indonesia Co., East Java, Indonesia). The control group (d_0) received feed at 3% of total fish biomass, while treatment groups were replaced with fermented *O. smaragdina* larvae at 10 (d_{10}), 15 (d_{15}), and 20% (d_{20}). The fermentation process involved *Lactobacillus* sp. at a concentration of 109 CFU/mL, mixed with 100g of ground *O. smaragdina* larvae. To support bacterial growth, 11mL of *Lactobacillus* sp. were mixed with 10mL of molasses. The mixture was placed in a 500mL sample bottle and fermented anaerobically at 33°C for 24 hours, with stirring every 12 hours, and maintained at a pH of 6. For the pellet formulation, 10– 20% of the pellet composition was replaced with the wet-fermented *O. smaragdina* larvae, according to the treatment dosage in the study. Given that pelletized into a diameter of 2mm can be simplified, the homogenized mixture was pelletized into 2mm pellets and oven-dried at 55°C. Four diets containing fermented *O. smaragdina* and pellets were prepared, with nutrient content, as shown in Table (1).

Feeding trial

Experimental fish were obtained from the Sumber Pasir Freshwater Fisheries Laboratory, Brawijaya University (Malang City, East Java, Indonesia). Before the feeding trial, *O. niloticus* was acclimated for 7 days under controlled conditions: a temperature of 27–29°C, a pH of 6–8, and a dissolved oxygen (DO) level of 6.5mg/ L.

480 fish (initial weight of 3.3 ± 0.04 g) were evenly and randomly assigned to 16 aquariums (30 × 30 × 30 cm), each containing 20L of water and stocked with 30 *O. niloticus* fry per aquarium. The 16 aquariums were assigned to each diet for four treatments (d_0, d_{10}, d_{15}, and d_{20}) and four duplicates. *O. niloticus* samples were fed twice daily (08:00 and 16:00) to apparent satiation for seven weeks. The water temperature was maintained at 27–29°C throughout the feeding trial.

Table 1. Proximate composition of experimental diets (% dry matter, kcal/g)

Ingredient	**d_0**	**d_{10}**	**d_{15}**	**d_{20}**	**Fermented *O. smaragdina***
Commercial Pellet (%)	100.00	90.00	85.00	80.00	-
Fermented Weaver Ant (%)	-	10.00	15.00	20.00	100.00
Proximate Composition					
Crude Protein (%)	27.24	28.95	31.02	32.25	59.89
Crude Lipid (%)	4.26	5.73	6.45	5.94	13.46
Crude Fiber (%)	6.44	8.50	9.23	10.28	20.03
Ash (%)	6.81	9.17	9.27	9.57	5.65
Moisture (%)	6.98	12.63	14.16	15.54	82.15
Carbohydrate (%)	48.27	45.64	44.02	41.96	1.97
Gross Energy (kcal/g)	392.07	404.88	416.75	410.43	473.65

Sampling

The fish in each aquarium were numbered and weighed in bulk to calculate the growth-related parameters. Five fish were measured and averaged weekly in each aquarium to assess growth performance and survival rate. The Health Research Ethics Commission of the Faculty of Medicine, Brawijaya University, authorized the study protocol and experimental protocols used in this work.

Proximate analysis of experimental diet composition

The chemical composition of test diets was analyzed using standard AOAC methods to ensure consistency and accuracy. Moisture content was determined by oven-drying at 105°C (AOAC method 930.15). Crude protein was measured using the Kjeldahl method (AOAC method 988.05), and crude lipid content was analyzed through Soxhlet extraction (AOAC method 920.39). Ash content was assessed by combustion at 550°C for 8 hours

(AOAC method 942.05), while crude fiber was determined using the enzymatic-gravimetric method (AOAC method 991.43). These analyses ensured precise characterization of the feed nutrient content.

Hematology

One fish was randomly selected from each aquarium weekly for hematology analysis. Blood sampling from *O. niloticus* was performed utilizing a disposable syringe pre-treated with 3.8% sodium citrate as an anticoagulant to inhibit clotting. Blood was extracted from the caudal region by inserting a needle at a 45° angle and withdrawing the plunger gently. The collected samples were subsequently transferred into Eppendorf tubes. Sampling was conducted through the caudal vein, located at the base of the fishtail, using a 1 mL syringe. The total erythrocyte and leukocyte counts were determined following the methodology established by **Blaxhall and Daisley (1973)**, which involved calculating the number of leukocytes per cubic millimeter (cells/mm^3). Hematocrit levels were assessed based on the method outlined by **Anderson and Siwicki (1993)**, which calculates the percentage of packed cell volume. Hemoglobin concentration was evaluated using the Sahli method, expressed in grams per deciliter (g/dL) **(Irzaman *et al.*, 2022)**.

Intestines *Lactobacillus* analysis

Bacterial sampling involved extracting intestinal samples from *O. niloticus* at three intervals: days 7, 21, and 35. The necropsy included dissecting the fish to obtain the intestine, which was then ground into a paste in a mortar, using approximately 1 gram of tissue for each sample. The homogenized intestinal samples were diluted. To prepare the physiological saline solution, 0.9g of sodium chloride (NaCl) was dissolved in 100mL of distilled water in an Erlenmeyer flask. Subsequently, 9mL of the saline solution was transferred to a test tube, wrapped in cotton and aluminum foil for sterility. 68.2g of selective MRSA medium was dissolved in 1000mL of distilled water in an Erlenmeyer flask and boiled to prepare the agar medium. The solution was then sterilized in an autoclave at 121°C and 1 atm for 30 minutes. Bacterial inoculation was performed using the spread plate method. Diluted samples (10^{-2}, 10^{-3}, and 10^{-4}) were homogenized with a vortex mixer, and one milliliter from each diluted sample was transferred to Petri dishes with 15- 20mL of MRSA agar medium and incubated upside down at 37°C for 24 hours.

Histology

Fish were fasted for 24 hours, and two fish from each aquarium were randomly selected. They were then anesthetized with Tricaine Methanesulfonate (75mg/ L) and subsequently dissected. The hindgut samples of 2 fish were removed and preserved in paraformaldehyde (4 %) to analyze hindgut histology and microbiota analysis, and observed 3 times (days 7, 21, and 35). The excised intestinal tissue was placed into a cassette containing distilled water. The tissue was dehydrated using paraffin or other appropriate substances to prepare tissue blocks. Water removal from the cells and tissue

was accomplished by immersing the samples in 95% ethanol. Tissue blocks were subsequently formed using a base mold to apply hot liquid paraffin at 70ºC. The tissue was sliced using a microtome. Staining was executed using hematoxylin-eosin (HE), specifically employing Mayer's hematoxylin and eosin. The stained preparations were then examined under a binocular microscope to measure the length and width of the intestinal villi in *O. niloticus*.

Nitrogen and water quality

Water samples for nitrogen analysis were collected weekly until day 35 of the rearing period. The ammonia nitrogen levels (NH_3-N) were determined using colorimetric methods in accordance with the standards set forth by the International Organization for Standardization (ISO), which specifies a manual spectrometric approach for quantifying ammonium in water samples. Nitrate nitrogen (NO_3-N) concentrations were measured spectrophotometrically using cadmium reduction by ISO standards. Additionally, *in situ* measurements of dissolved oxygen (DO) and pH were conducted utilizing a dissolved oxygen meter (Model HI9146-04N, Hanna Instruments, Canada) and a portable pH meter (Model Hi9811-51, Hanna Instruments, Canada).

Calculations and statistics

The following techniques were used to calculate the growth-related parameters:

Feed intake (FI, g/ind) = consumed feed weight (dry weight)/fish number.

Weight gain rate (WGR, %) = 100 × (final fish weight-initial fish weight)/initial fish weight

Specific growth rate (SGR, %/d) = 100 x [ln (final fish weight)-ln (initial fish weight)] / feeding days

Feed conversion ratio (FCR) = body weight gain / FI

Condition factor (CF, g/cm^3) = body weight/body length^3

Survival rate (SR, %) = 100 × final fish number/initial fish number

Data are presented as mean ± standard deviation and were analyzed using SPSS 16.0 software with one-way analysis of variance (ANOVA). Duncan's multiple range test was applied for post-hoc comparisons when ANOVA indicated significant differences (P< 0.05).

RESULTS

Length and width of the villus

Histological analyses focused on measuring the length and width of the intestinal villi in *O. niloticus* (Fig. 2), with dimensions assessed as shown in Fig. (2g). The intestine plays a vital role in digestion, particularly in nutrient absorption, with villi being key structural components **(Bušelić *et al*., 2025)**. Longer and wider villi correlate with an increased surface area, enhancing nutrient absorption efficiency **(Ramos *et al*., 2017)**.

Among the tested dosages (Fig. 2a), the d15 treatment (81.5µm) significantly increased villus width, particularly after day 20, reaching 38.10µm. In contrast, the d_0 group (43.62µm) exhibited lower villus width than both d_{15} (94.6 µm) and, at times, d_{10} (47.6µm). The 20% dosage (81.5µm) also improved villus width but was less effective than d_{15}. Regarding villus length (Fig. 2b), d_{15} (179.64µm) had the most significant impact, especially after day 20 (173.45µm). While d_{20} (178.17µm) showed some increase, it was slightly lower than d_{10} (185.00µm) and less effective than d_{15}. Overall, the d_{15} dosage was deemed the most effective, promoting optimal villus development without adverse effects. In contrast, d_{10} was less effective, and d_{20} did not yield comparable improvements. The high dosage may have induced intestinal inflammation, likely due to the increased crude fiber content in the feed, as evidenced in Fig. (2e, f).

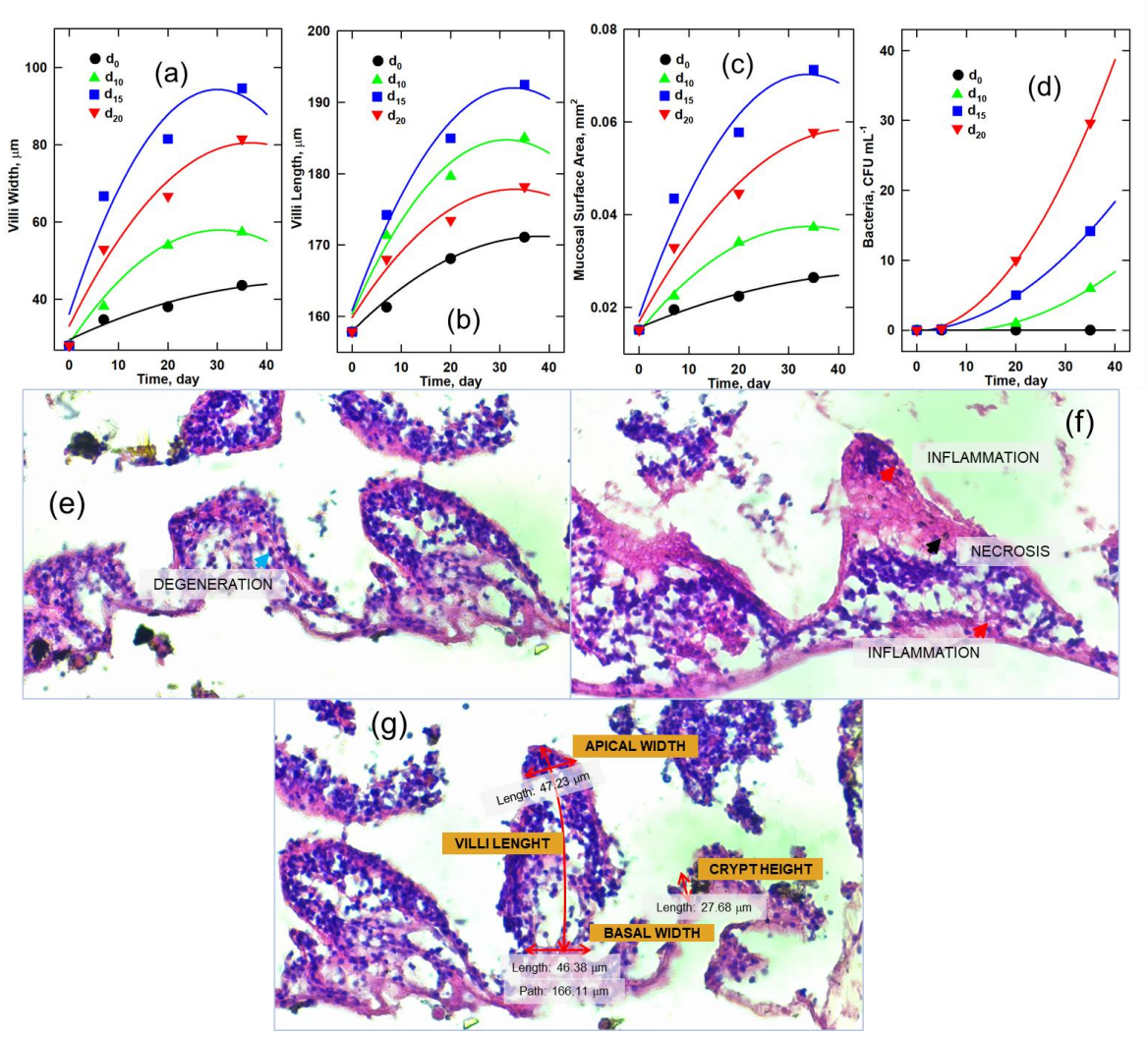

Fig. 2. Intestinal observations of *O. niloticus* following *Lactobacillus*-fermented *O. smaragdina* larvae dosage on (a) villus width, (b) villus length, (c) mucosal surface area, and (d) abundance of *Lactobacillus* bacteria. Comparison of the intestinal conditions of *O. niloticus* in doses (e) d_0 and (f) d_{20}, as well as (g) measuring the dimensions of villi

Abundance of *Lactobacillus* bacteria in the gut

Incorporating *Lactobacillus* bacteria into feed enhances its digestibility, which is attributed to the enzymes that break down complex compounds into simpler substances during fish digestion **(Jiang *et al.*, 2023)**. *Lactobacillus* acts as a beneficial bacterium, preventing pathogenic bacteria in the intestinal tract and metabolizing carbohydrates into lactic acid **(Huang *et al.*, 2022)**. By day 7, bacterial density had emerged in the dosage groups (Fig. 2d), contrasting with the control at day 0, which showed a density of 2 CFU/mL. An initial increase was noted at the d_{10} (0.069 x 10^6 CFU/mL), with more significant growth in higher dosages: d_{15} (0.14 x 10^6 CFU/mL) and d_{20} (0.197 x 10^6 CFU/mL). These results indicate that higher dosages accelerate bacterial growth in the intestines. By day 35, bacterial density peaked in the d_{20} at 29.64 x 10^6 CFU/mL, followed by d_{15} (14.16 x 10^6 CFU/mL) and d_{10} (5.95 x 10^6 CFU/mL). In contrast, the d_0 (891 CFU/mL) showed no growth, and higher dosages (d_{15} and d_{20}) led to faster bacterial proliferation, enhancing digestion and offering earlier protection against pathogens, ultimately supporting better fish growth compared to lower dosages or the absence of *Lactobacillus* supplementation.

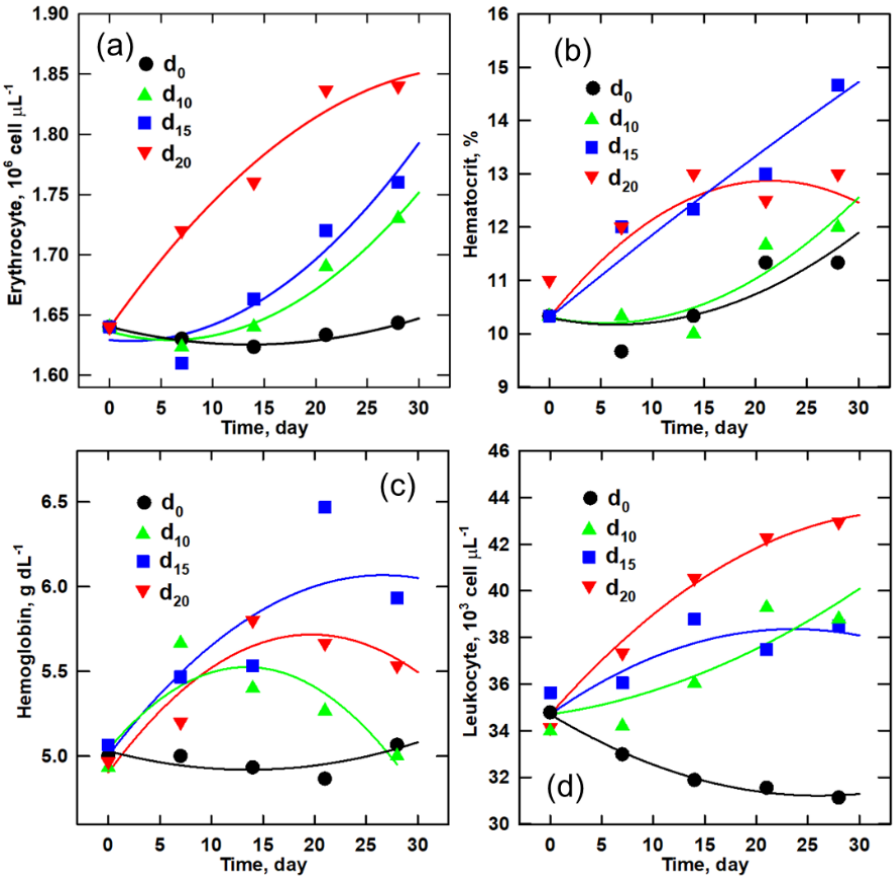

Fig. 3. The effect of *Lactobacillus*-fermented *O. smaragdina* larvae supplementation on (a) erythrocytes, (b) hematocrit, (c) hemoglobin, and (d) leukocytes in *O. niloticus* at each termination day

Leukocytes

Leukocytes are critical indicators of infection in *O. niloticus* **(Sherif *et al.,* 2020)**. The body increases leukocyte production in response to foreign substances, reflecting heightened cell division **(Fazio *et al.,* 2019)**. In healthy *O. niloticus*, leukocyte counts range from 20,000 - 150,000 cells/mm^3 **(Lestari *et al.,* 2020)**. As shown in Fig. (3a), the d_{20} consistently increases leukocyte counts from day 0 (34.13 x 10^3 cells/mm^3) to day 28 (42.95 x 10^3 cells/mm^3). In contrast, the d_{10} starts increasing on day 7, peaks on day 21 (39.28 x 10^3 cells/mm^3), and slightly declines to day 28 (38.80 x 10^3 cells/mm^3). The d_0 shows a significant decrease in leukocyte levels from 34.78 x 10^3 cells/mm^3 to 31.13 x 10^3 cells/mm^3 by day 28. The d_{15} fluctuates, peaking at 38.78 x 10^3 cells/mm^3 on day 14 before declining. The consistent increase in the d_{20} dosage suggests that the fish may have experienced stress or infection, requiring environmental adaptation. In contrast, the fluctuating levels in the d_{10} and d_{15} dosages indicate a more effective adaptation process, with leukocyte counts decreasing after their peak. The significant decline in the d_0 suggests the fish were healthy, marked by low stress and minimal adaptation needs.

Hemoglobin

Hemoglobin (Hb) levels are crucial for the oxygen-binding capacity of red blood cells **(Wang *et al.,* 2022)**. Low Hb can lead to decreased metabolic rates, reduced energy production, weakness, and decreased appetite in fish **(Obirikorang *et al.,* 2020)**. Observations in Fig. (3b) show that hemoglobin levels in the d_0 remained stable at approximately 4.9 - 5.0 G% throughout the observation period. The d_{10} dosage experienced a slight increase by day 7 (around 5.67 G%), followed by minor fluctuations. The d_{15} showed a notable increase, peaking at approximately 6.47% on day 21, while the d_{20} dosage had similar trends but with less pronounced volatility. The d_{15} dosage was the most effective in enhancing hemoglobin levels in *O. niloticus*, approaching the healthy range of 6 - 11 G%, supporting optimal metabolic function **(Haque *et al.,* 2021)**. Although the d_{10} dosage showed a slight increase, it was insufficient to reach ideal levels, contributing to reduced metabolism, energy, and appetite **(Hans *et al.,* 2018)**. The d_{20} group also indicated an increase, though not as substantial as the d_{15} group.

Erythrocytes

Erythrocytes containing hemoglobin are essential for oxygen transport throughout the body **(Witeska *et al.,* 2022)**. Appetite significantly affects erythrocyte counts; fish with reduced appetite often exhibit lower levels **(Shen *et al.,* 2018)**. In teleosts, erythrocyte counts typically range from 1.05 - 3.0 x 10^6 cells/mm^3 **(Nabi *et al.,* 2022)**. Increasing the total number of erythrocytes is believed to be a response to stress in fish. As depicted in Fig. (3c), the d_0 group showed a stable increase in erythrocyte counts, rising from 1.64 x 10^6 cells/mm^3 on day 0 to 1.84 x 10^6 cells/mm^3 on day 28. The d_{10} displayed a relatively flat trend, with a slight increase on day 7 (1.63 x 10^6 cells/mm^3) and a gradual rise to 1.643 x 10^6 cells/mm^3 by day 28. The d_{20} group significantly increased

on days 21 (1.72 x 10^6 cells/mm^3) and 28 (1.76 x 10^6 cells/mm^3). The d_{15} dosage showed minor fluctuations but ultimately reached 1.73 x 10^6 cells/mm^3 by the end of the observation period. The d_{15} dosage was the most effective in increasing erythrocyte counts and improving oxygen-binding capacity and metabolism.

Hematocrit

Hematocrit is the percentage of red blood cells in the blood **(Bavia *et al.*, 2024)**. In teleosts, typical hematocrit values range from 20 - 30%, while fish aged three weeks typically exhibit values between 10 and 20% **(De *et al.*, 2019)**. Observations in Fig. (3d) indicate that the d_0 had a slight increase from day 0 (10.33%) to day 28 (11.33%). The d_{10} showed a more stable increase, rising from 10.33 to 12% over the same period. In contrast, the d_{15} group showed a significant increase, rising from 10.33% to 14.66%. The d_{20} dosage also increased from 11 to 13%, a more significant increase than in other groups. The d_{15} dosage showed the most significant increase in hematocrit, indicating improved oxygen transport capacity, metabolism, and stress resilience in the fish **(Anttila *et al.*, 2023)**. The d_{20} dosage yielded favorable results, while the d_0 and d_{10} dosages showed fewer notable increases but remained within a safe range.

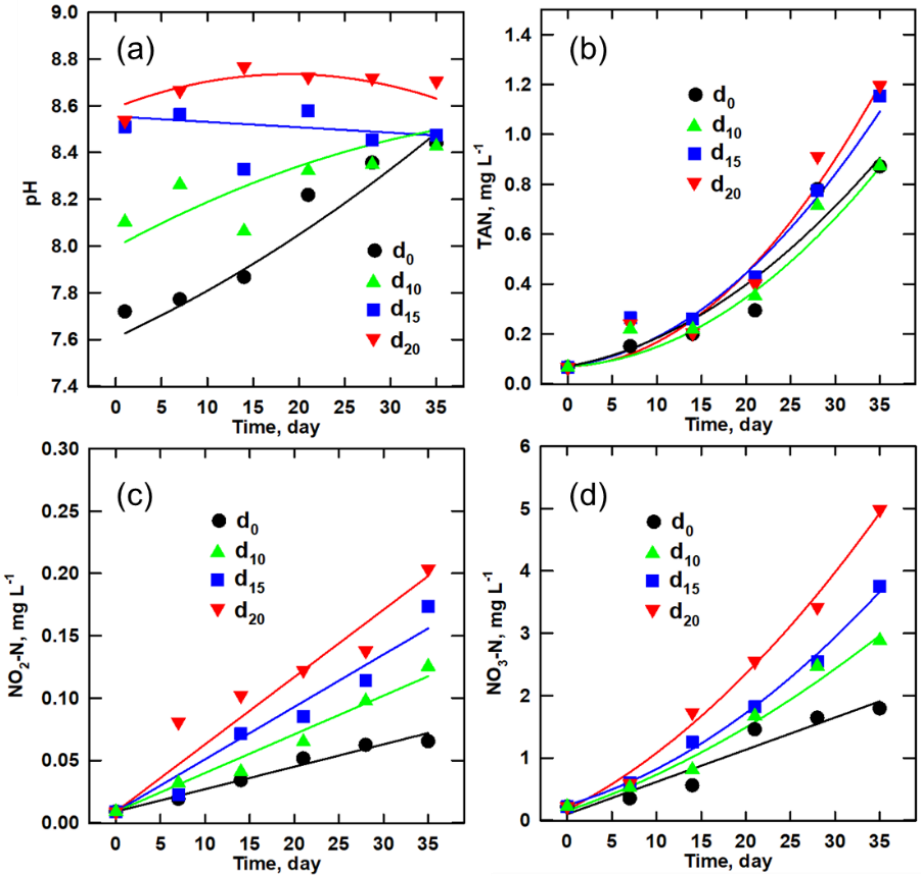

Fig. 4. The Effect of Feeding *Lactobacillus*-fermented *O. smaragdina* larvae on (a) pH and Nitrogen (in the form of (b) TAN, (c) NO_2, and (d) NO_3) in the culture medium for *O. niloticus* on each observation day

pH

pH is a crucial water quality parameter that reflects hydrogen ion activity and influences microbial life and aquatic fertility **(Edwards *et al.*, 2024; Sholichin *et al.*, 2024)**. Low pH values can harm fish, while an optimal pH range for *O. niloticus* is between 6 and 8.5 **(Pellegrin *et al.*, 2020; Rahardjo & Shih, 2023)**. Higher dosages generally correlate with elevated pH values, as shown in Fig. (4a). The control (d_0) pH increased from 7.72 to 8.35 over the observation period. The d_{10} started at 8.10 on day 7 and rose to 8.35 by day 28, with slight fluctuations. The d_{15} exhibited minor fluctuations, decreasing from 8.51 to 8.45 by day 35. The d_{20} dosage consistently increased, rising from 8.53 to 8.72, surpassing the d_0 pH of 8.4. Exceeding the upper limit of 8.5 can diminish productivity and increase stress risks in fish, thereby affecting the balance of dissolved oxygen.

Total ammonia nitrogen

TAN (NH_4/NH_3) levels are considered toxic for short-term exposure when exceeding 0.6-2.0 mg/L, posing risks to various fish species **(Rahardjo & Shih, 2023)**. Ammonia is a metabolic byproduct of *O. niloticus*, and its concentration is influenced by pH levels **(Rahardjo & Shih, 2022)**. By day 7, TAN levels rose across all dosages (Fig. 4b), with d_{15} showing the highest concentration (0.26mg/ L), followed by d_{20} (0.24mg/ L), d_{10} (0.22mg/ L), and d_0 (0.15mg/ L). By day 21, all groups showed significant increases, particularly the d_{15} (0.43mg/ L) and d_{20} (0.40mg/ L). A notable spike occurred on day 28, with the d_{20} dosage reaching 0.91mg/ L and the d_{15} at 0.77mg/ L, while the d_{10} and d_0 remained lower. By day 35, TAN peaked at 1.20mg/ L for the d_{20} and 1.15mg/ L for the d_{15}, indicating that higher dosages lead to faster TAN accumulation, posing risks in aquaculture.

Nitrite

Nitrite (NO_2^-) is typically present in smaller quantities than nitrate (NO_3^-), due to its instability in oxygen-rich environments **(Ao *et al.*, 2024)**. As an oxidized nitrogen form, nitrite can be lethal to aquatic organisms, with concentrations above 0.2mg/ L posing hazards to fish **(Rahardjo & Shih, 2024)**. In the d_0, nitrite only reached 0.065mg/ L by day 35, indicating a slow increase, as shown in Fig. (4c). The d_{10} dosage rose to 0.125mg/ L, while the d_{15} dosage peaked at 0.174mg/ L after day 28. The d_{20} group had the highest nitrite concentration at 0.204mg/ L by the end of the observation period, with significant increases starting on day 7. Higher dosages markedly influenced nitrite levels, particularly in the d_{15} and d_{20} groups, raising concerns for fish health.

Nitrate

Nitrate compounds are the predominant form of nitrogen in aquatic environments and are essential for the growth of plants and algae **(Wu *et al.*, 2023)**. The minimum tolerance for nitrate-related algal growth is 0.1mg/ L, while levels above 10mg/ L can

restrict fish farming **(Dauda *et al.*, 2019)**. In the d_0, nitrate rose slowly from 0.22 to 1.64mg/ L by day 28 (Fig. 4d). The d_{10} increased from 0.22 to 2.47mg/ L, while the d_{15} reached 2.54mg/ L by day 28. The d_{20} showed the most significant increase, peaking at 3.42mg/ L by day 28. Nitrate concentrations are significantly influenced by dosage; higher dosages lead to more pronounced increases, particularly evident in the d_{20} after day 14.

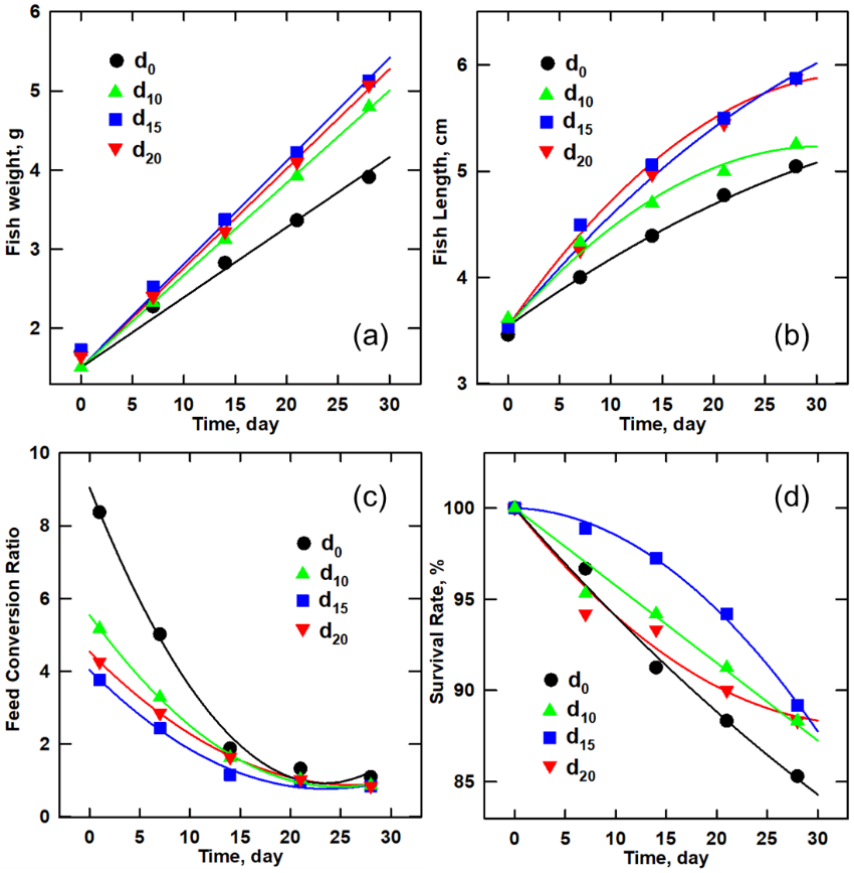

Fig. 5. Impact of feeding Lactobacillus-fermented *O. smaragdina* larvae at different doses on (a) weight, (b) length, (c) FCR, and (d) survival rate of *O. niloticus* at each observation day

Weight and length of fish

Growth is characterized by changes in length and weight over time, reflecting an increase in cell numbers through mitosis and resulting in alterations to tissue size **(Mahalder *et al.*, 2023)**. The weight of fish (Fig. 5a) in the control d_0 increased steadily from 1.69g on day 0 to 3.91g on day 28, but this rate was slower than other dosages. The d_{10} showed a more rapid growth, rising from 1.505 to 4.8g. The d_{15} resulted in the most significant weight gain, increasing from 1.73 grams to 5.13g, making it the most effective dosage. The d_{20} also displayed considerable growth, from 1.64 to 5.08g, nearly matching the d_{15} results. On day 28, the d_{15} had the highest length at 5.875cm, closely followed by d_{20} at 5.873cm, as shown in Fig. (5b). Both dosages achieved similar length

enhancements. The d_{10} dosage yielded a length of 5.25cm, which was slightly lower than the 15 and 20% dosages. In contrast, the d_0 had the slowest growth, reaching only 5.05cm.

Survival rate

The survival rate of *O. niloticus* is influenced by factors such as water quality, feed, and fish health **(Ibrahim *et al.,* 2023)**. In well-managed farming conditions, survival rates can reach 80- 90% **(Clyde *et al.,* 2023)**. The d_{15} achieved the highest survival rate at each observation, particularly on day 28 (89.17%). The d_{20} and d_{10} dosages showed similar survival rates of 88.33%, with d_{10} performing slightly better in the early observations (days 7 to 14). The d_0 dosage had the lowest survival rate decline, indicating that the absence of dosing may be suboptimal, yet it remained acceptable for practical *O. niloticus* farming.

Feed conversion ratio (FCR)

The FCR measures the amount of feed required to produce 1kg of *O. niloticus* **(Verdal *et al.,* 2022)**. It is calculated by evaluating the total feed provided and the final and initial biomass. FCR values varied among treatments, as shown in Fig. (5c). On day 1, the d_0 had the highest FCR (8.37), while d_{20} had a lower FCR (4.26), indicating that untreated fish converted feed less efficiently. A significant reduction in FCR was observed by day 7, particularly for d_0, which decreased to 5.02, and for d_{20}, which decreased to 2.85. By day 14, the FCR improved, with d_0 at 1.88 and d_{20} at 1.64. The d_{15} dosage showed the lowest FCR (1.15), indicating the best feed efficiency. On days 21 and 28, all groups had low FCR values, with d_{15} and d_{20} performing best at 0.97 and 0.84, respectively, on day 28. The dosages, particularly d_{10}, d_{15}, and d_{20}, had a positive influence on feed efficiency, with d_{15} and d_{20} showing the most significant enhancements in feed conversion efficiency.

DISCUSSION

The feeding of fermented ant larvae significantly affected the length and width of the villi in *O. niloticus* and the density of intestinal *lactobacillus* ($P<0.05$), as shown in Table (2). However, Duncan's test revealed an inconsistency between villi size and *Lactobacillus* sp. density at the d_{20}. Although a higher *lactobacillus* sp. The density was expected to enhance nutrient absorption; the villi size was smaller than that recorded in the control (d_0). In contrast, the 15% dosage showed a positive correlation between villi size and *lactobacillus* density, with both metrics increasing concurrently. Moreover, increasing the feed dosage had a positive impact on the fish's intestinal condition. The 15% dosage significantly enhanced the length and width of the intestinal villi, resulting in a larger surface area for nutrient absorption. This increase allows the fish to utilize feed more efficiently, contributing to overall weight and length gains.

Table 2. Average observations of growth, hematology, intestinal condition, and nitrogen at each dosage, along with the results of variance analysis

Parameter	Dosage			
	d_0	d_{10}	d_{15}	d_{20}
Fish Growth				
Weight, g	3.913 ± 0.118^{a}	4.8 ± 0.316^{b}	5.125 ± 0.655^{b}	5.075 ± 0.809^{b}
Length, cm	5.045 ± 0.0265^{a}	5.253 ±0.052^{ab}	5.875 ± 0.696^{b}	5.873 ± 0.627^{b}
SR, %	85.433 ± 0.808^{a}	87.96 ± 0.340^{a}	89.613 ± 0.767^{a}	87.3 ± 1.001^{a}
FCR	1.095 ± 0.0121^{b}	0.865 ± 0.029^{ab}	0.842 ± 0.117^{a}	0.844 ±0.089^{a}
FI, g/Ind	4.283 ± 0.437	4.151 ± 0.497	4.315 ± 0.335	4.283 ± 0.590
WGR, %	66.450 ± 3.763	98.850 ± 2.452	101.925 ± 8.594	102.975 ± 4.577
SGR, %/d	1.561 ± 0.251	2.079 ± 0.165	2.007 ± 0.382	2.135 ± 0.063
CF, g/cm^3	0.030 ± 0.001	0.033 ± 0.003	0.026 ± 0.006	0.026 ± 0.006
Hematology				
Erythrocytes, 10^6 cell L^{-1}	1.640 ± 0.044^{a}	1.666 ± 0.065^{a}	1.697 ± 0.029^{ab}	1.759 ± 0.0208^{b}
Hemoglobin, g dL^{-1}	4.973 ± 0.046^{a}	5.173 ± 0.289^{a}	5.693 ± 0.180^{b}	5.293 ±0.129^{a}
Leukocytes, 10^3 cell L^{-1}	32.467 ± 2.190^{a}	36.46 ± 2.528^{b}	37.293 ± 3.607^{b}	40.643 ± 0.917^{c}
Hematocrit, %	10.6 ± 0.872^{a}	11.2 ± 0.2^{a}	12.867 ± 0.416^{b}	12.25 ± 0.477^{b}
Intestines				
Villi Width, mm	35.537 ± 0.790^{a}	47.323 ± 1.655^{b}	79.02 ± 2.91^{c}	49.47 ± 0.120^{b}
Villi Length, mm	166.233 ± 2.503^{a}	177.067 ± 0.950^{b}	182.467 ± 2.450^{c}	168.3 ± 1.8^{a}
Abundance of bacteria, Log CFU mL^{-1}	2.950 ± 0.073^{a}	6.775 ± 0.036^{b}	7.151 ± 0.207^{bc}	7.472 ± 0.021^{c}
Nitrogen appearances				
TAN, mg L^{-1}	0.923 ± 0.009^{a}	0.927 ± 0.006^{a}	1.223 ± 0.015^{b}	1.267 ± 0.025^{b}
Nitrite, mg L^{-1}	0.216 ± 0.005^{a}	0.414 ± 0.003^{b}	0.575 ± 0.007^{c}	0.674 ± 0.007^{d}
Nitrate, mg L^{-1}	1.793 ± 0.187^{a}	2.887 ± 0.086^{b}	3.757 ± 0.070^{c}	4.983 ± 0.110^{d}
pH	7.836 ± 0.070^{a}	8.16 ± 0.056^{b}	8.416 ± 0.059^{c}	8.57 ± 0.060^{c}

Hematological parameters, including erythrocyte counts, hemoglobin levels, and hematocrit percentages, showed significant differences among the various dosages. Duncan's analysis indicated that dosages above 20% performed poorly compared to the control (0%), suggesting that fish at these higher dosages experienced elevated stress

levels, which may lead to anemia. Conversely, the 15% dosage was optimal for fish health, indicating that fish receiving this dosage had a greater capacity for oxygen transport, thereby supporting metabolic functions and growth. Elevated hemoglobin and hematocrit levels facilitate better oxygen absorption and distribution, promoting faster growth rates **(Jin *et al.*, 2024)**.

Water quality, particularly TAN, nitrite, and nitrate levels, is influenced by feed dosage. Higher dosages, such as 15% and 20%, increase TAN and nitrite accumulation, potentially causing stress and reduced growth if not properly managed **(Isaza *et al.*, 2018)**. The rise in pH values at higher doses underscores the importance of rigorous water quality monitoring to maintain optimal conditions for fish **(Lemos *et al.*, 2018)**. Increasing the feed dosage significantly enhances weight, length, and FCR, as shown in the matrix correlation in Fig. (6), with d_{15} proving the most effective. Although d_{20} shows similar growth results, it also increases toxic factors, such as TAN and nitrite, which can harm the environment if left uncontrolled. Therefore, balancing optimal growth-supporting feed with effective water quality management is essential. Monitoring pH, TAN, nitrite, and nitrate levels is crucial to avoid harmful conditions, especially at dosages above 15%. Regular fish health assessments of the impact on water quality, particularly leukocyte counts and hemoglobin levels, are vital for maintaining fish well-being throughout their growth.

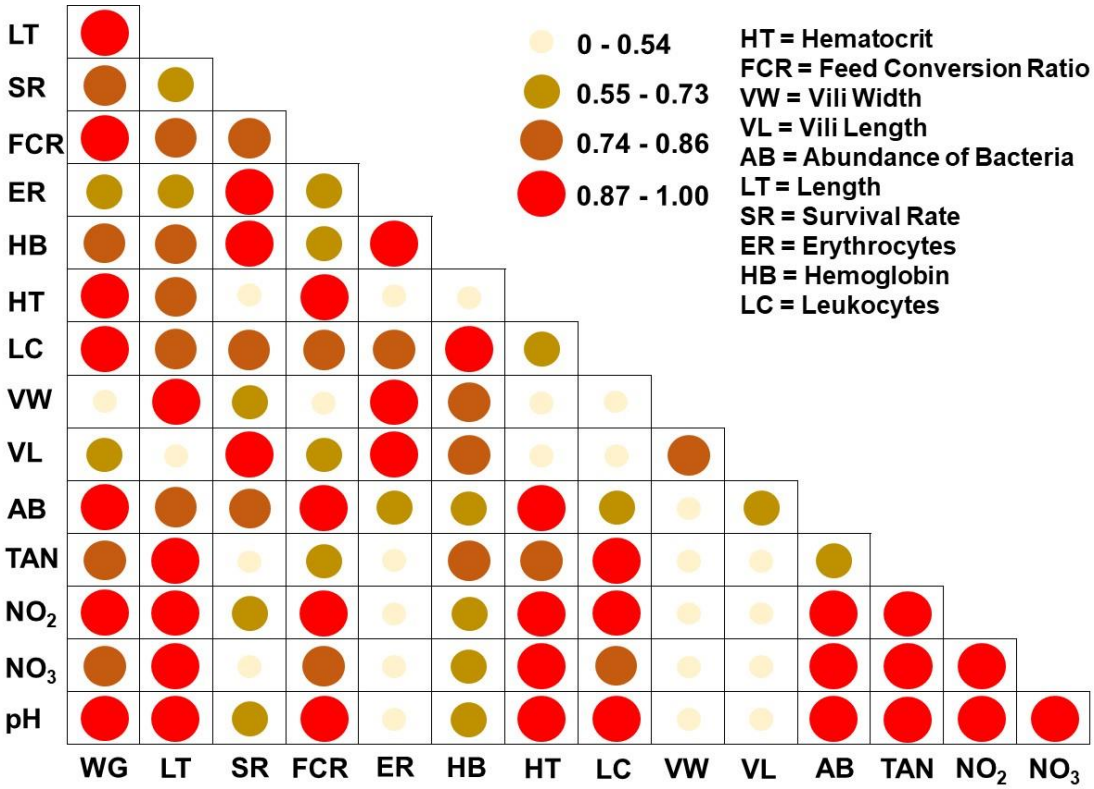

Fig. 6. Correlation matrix of parameters following the dosage

CONCLUSION

This study highlights the potential of *Lactobacillus*-fermented *Oecophylla smaragdina* larvae as an effective feed supplement for *O. niloticus* during the nursery phase. The 15% dosage emerged as optimal, significantly improving weight gain, length growth, survival rate, and feed conversion efficiency while maintaining favorable water quality and fish health. Enhanced hematological parameters and intestinal villus morphology in the d_{15} group reflect improved nutrient absorption and physiological resilience. While the 20% dosage demonstrated similar growth benefits, it increased TAN and nitrite levels, posing risks to water quality and fish well-being. The findings underscore the importance of balancing feed supplementation with environmental management, making d_{15} a practical and sustainable choice for aquaculture.

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