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# Effects of biosynthesized zinc oxide nanoparticles as feed additives on growth and hematological parameters of striped dwarf catfish (*Mystus vittatus*)

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ABSTRACT

Striped dwarf catfish *(Mystus vittatus*) is a valued freshwater species in South Asia for its taste, nutritional profile, and year-round availability. This study evaluated the effects of biosynthesized zinc oxide nanoparticles (ZnONPs) on the growth performance and hematological parameters of *M. vittatus*. A total of 960 fish (80/aquarium) were reared in twelve aquariums, and fed diets containing ZnONPs at 0 ($T_0$), 50 ($T_1$), 80 ($T_2$), and 110 ($T_3$) mg/kg for 75 days. Treatment $T_3$ showed the highest weight gain (233.33%), specific growth rate (1.60%/day), and protein efficiency ratio (2.01±0.002), along with the lowest feed conversion ratio (FCR) ($p<0.05$). Hematological parameters (RBC, Hb, WBC, HCT, etc.) varied numerically, but statistical tests (ANOVA and Kruskal–Wallis) showed no significant differences ($p>0.05$). Values at 80 mg/kg appeared more stable, though not statistically validated. Effect size analysis indicated large variance explained by treatment for key indices ($\eta^2 = 0.31–0.39$ for RBC, WBC, Hb, HCT). These values suggest biologically meaningful differences that were not statistically confirmed, likely due to small sample size. By contrast, indices with low effect sizes showed minimal treatment influence, indicating real stability across diets. The survival rate (67.50±0.73%) improved with increasing ZnONPs concentrations, reaching the highest in $T_3$ ($p < 0.05$). Although mortality was a bit higher, likely due to artificial aquarium conditions and early-stage sensitivity, ZnONPs supplementation still showed a positive effect. These findings suggest that dietary ZnONPs at 110 mg/kg improved growth and survivability in *M. vittatus* without adverse hematological effects.

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## Introduction

Modernization of the aquaculture industry promises increased biomass production utilizing advanced techniques to meet the nutritional requirements of the growing population (Boyd *et al.*, 2020). However, the industry encounters significant economic losses due to the emergence of infectious diseases caused by various pathogens (Patil *et al.*, 2025). The application of antibiotics can be considered an efficient technology for instant solutions to the drawback. Still, they can lead to the emergence of antibiotic-resistant pathogens and environmental contamination as the main receptors of antibiotics can last even after the treatment and are discharged into the environment (Romero *et al.*, 2012; Michael *et al.*, 2013). Utilization of probiotics, prebiotics, plant extracts, and synbiotics poses an alternative measure of antibiotics in fish due to their growth performance, survival rate, and immune response-enhancing attributes (Harikrishnan *et al.*, 2019; Rohani *et al.*, 2022). However, most commercial probiotics available on the market are non-native to many countries, including Bangladesh, Pakistan, and Myanmar, and their effectiveness is often dependent on specific species and environmental conditions (Hossain *et al.*, 2023). Therefore, adequately efficient techniques should be introduced across all fish species and environments. One such promising approach is the use of nanoparticle-based technology. This technology offers several advantages, including enhanced bioavailability, lower dosage requirements, reduced antibiotic residues, and decreased environmental contamination (Rakhi *et al.*, 2022). Moreover, they suggested that nanoparticle-based trace minerals can meet fish dietary mineral requirements more effectively. Nanoparticles can modulate key physiological functions across aquaculture species. For example, dietary silver nanoparticles (10–15 µg/kg) improved growth, immune-gene expression, and resistance to *Aeromonas hydrophila* in rohu (*Labeo rohita*) (Popoola *et al.*, 2023). Likewise, selenium nanoparticles (~1 mg/kg) enhanced growth performance and improved fillet fatty-acid profiles in Nile tilapia (*Oreochromis niloticus*) (Moges *et al.*, 2022). More recently, green-synthesized ZnO nanoparticles (≈60 mg/kg) promoted growth, intestinal integrity, and immune responses in Nile tilapia (Alafnan *et al.*, 2024). In another study, Shahariar *et al.* (2024) suggested 30 mg/kg gold nanoparticles as a promoting agent of growth, survivability, and hematological parameters in striped dwarf catfish (*M. vittatus*).

Among various nanoparticles, ZnONPs are extensively known and used for their efficacy in improving fish health and growth (Asad *et al.*, 2024). Moreover, they are non-toxic, biocompatible, drug carriers, and antipathogenic in nature (Krithika *et al.*, 2017). These outstanding features led the researcher to utilize ZnONPs as feed additives for several fish and aquatic species (Faiz *et al.*, 2015; Kumar *et al.*, 2022, 2023). Thangapandiyan and Monika (2019) reported a positive correlation of growth and metabolic activity with the rising

concentration of dietary ZnONPs in rohu (*L. rohita*). ZnONPs at 20 mg/kg was shown to be effective in growth promotion, oxidative stress relief, and immunity improvement (Kumar *et al.*, 2018), while 30 mg/kg ZnONPs-enriched diet supported better growth and protein utilization in *Siganus rivulatus* (Sallama *et al.*, 2020). Moreover, dietary supplementation of zinc oxide nanoparticles (ZnONPs) at 30 and 60 µg/g feed enhanced the resistance of Nile tilapia (*O. niloticus*) against *A. hydrophila* infection, resulting in a significantly higher survival rate (Sherif *et al.*, 2023).

Striped dwarf catfish (*M. vittatus*) typically inhabits marginal vegetation zones in lakes and swamps with muddy substrates, and demonstrates omnivorous feeding behavior, consuming a variety of plants, insect larvae, shrimps, mollusks, zooplankton, and small fish (FishBase; Gupta, 2014).This species is valued for its palatable taste and rich nutritional profile, including protein, micronutrients, vitamins, and minerals that are often limited in other foods (Molla *et al.*, 2008). It was previously categorized as a vulnerable species due to a 36.6% decline in catch from natural habitats in Bangladesh between 1960 and 2000. While the striped dwarf catfish (*M. vittatus*) is currently classified as Least Concern by the IUCN, evidence indicates that its population size and range are gradually declining, necessitating management interventions to ensure its long-term survival in the wild (Mawa *et al.*, 2022). In addition, this small indigenous species, locally known as "Tengra", is increasingly recognized for its aquaculture potential, particularly in polyculture systems within seasonal ponds, where it performs well under short-cycle farming practices (Hasan *et al.*, 2023). Therefore, this study was designed to evaluate the effects of biosynthesized ZnO nanoparticles as dietary supplements on the growth performance, survivability, and hematological responses of *M. vittatus*.

## Materials and Methods

### *Experimental diet formulation*

The experimental diets were prepared by incorporating biosynthesized ZnONPs into a commercial feed (Mega Feed, Spectra Hexa Feeds Limited, Code No. 001) which, according to the feed packet, contained approximately 37% protein, 24% carbohydrate, 8% lipid, 16% ash, 12% moisture, 4% fiber, 2.1% calcium, and 0.8% phosphorus, (values given as approximate maximum levels), with a pellet diameter of 0.6 mm. ZnONPs were added at concentrations of 0 ($T_0$, control), 50 ($T_1$), 80 ($T_2$), and 110 ($T_3$) mg/kg of feed. Biosynthesized ZnONPs, derived from seaweed extract, were obtained from the Department of Plant Pathology, Gazipur Agricultural University. Initially, all feeds ($T_0$, $T_1$, $T_2$, and $T_3$) were ground into powder to ensure homogeneous mixing. For proper mixing with the commercial diet, the ZnONPs were blended with deionized water using an ultrasound bath (Elma_17013, Elma Schmidbauer, Germany) for 10 min at 37 KHz (Shahariar *et al.*, 2024). The ZnONPs-water mixture was then added to the feed powder (except for $T_0$), pelleted, and sun-dried. The

prepared diets were stored under refrigeration at approximately 4-10˚C until use.

### *Experimental design*

The experimental fish were collected from a hatchery one week before the experiment and were acclimatized to the tank conditions at the experimental site to minimize handling and transport stress. The feeding trial was carried out in 12 rectangular glass aquariums (60 cm × 45 cm × 45 cm), each holding 70 liters of water (Afrin *et al.*, 2023). Each treatment was replicated three times, with individual aquariums serving as replicates. Thus, the experimental unit was the tank (n = 3 per treatment), not individual fish. Among them, only the control group (T0) received the commercial diet containing 0 mg/kg of biosynthesized ZnONPs whereas the other three groups ($T_1$, $T_2$, and $T_3$) were provided diets supplemented with 50, 80, and 110 mg/kg of biosynthesized ZnONPs, respectively. Initially, 80 fish were weighed and placed in each aquarium. The fish were fed three times daily (early morning, afternoon, and late night) until satiation. Approximately 30% of the water in each aquarium was replenished weekly. Each aquarium was continuously aerated with an air stone connected to a central air compressor.

### *Water quality monitoring*

Temperature, dissolved oxygen (DO), ammonia, pH, and total dissolved solids (TDS) in each aquarium were measured at 9:00 a.m. every five days throughout the study period.

### *Fish growth monitoring and sampling design*

Every fifteen days, 20 fish from each aquarium were randomly caught using a scoop net, and their body weight and length were recorded for growth performance analysis. Measurements from the 20 fish were averaged within each tank at each sampling point. These tank means were then used in the statistical analysis, ensuring that replication was based on the three tanks per treatment. Using formulas from earlier studies (Shahariar *et al.*, 2024), the growth parameters and survival rate were calculated:

$$\text{Weight Gain (WG\%)} = \frac{\text{Mean final fish weight - Mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$$

$$\text{Specific Growth Rate (SGR\%/day)} = \frac{\text{In (Mean final fish weight) - In (Mean initial fish weight)}}{\text{No of days of the experiment}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed consumption}}{\text{Total body weight gains of fish}} \times 100$$

$$\text{Protein Efficiency Ratio (PER \%)} = \frac{\text{Total weight gain (g)}}{\text{Amount of protein intake (g)}} \times 100$$

$$\text{Condition Factor (g/cm}^3\text{), CF} = \frac{\text{Mean final body weight}}{\text{Mean final body length}^3} \times 100$$

$$\text{Survival Rate (SR\%)} = \frac{\text{No of the live fishes}}{\text{No of the fish introduces}} \times 100$$

### *Analysis of hematological parameters*

At the end of the 75-day trial, approximately 10–15 fish were randomly sampled from each tank for blood collection. Owing to the small size of fish, this number varied slightly between tanks. Blood indices from fish within a tank were averaged to generate one tank-level value, with tanks treated as the experimental unit (n = 3 per treatment). After removing excess water with a dry cloth, blood was collected from the caudal peduncle region using a sterile syringe and transferred into an EDTA (Ethylenediaminetetraacetic Acid) tubes. Hematological parameters were analyzed immediately using a fully automated hematology analyzer (Mindray BC-2300).

### *Statistical data analysis*

All statistical analyses were performed using R software (version 4.3.1; R Core Team, 2023). Prior to analysis, the dataset was checked for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test (*car* package). When the assumptions of normal distribution and equal variance were satisfied, the data were analyzed by one-way analysis of variance (ANOVA) to evaluate the effects of dietary treatments. In cases where significant differences were observed, Tukey's Honestly Significant Difference (HSD) test was applied as a post hoc procedure to separate means at a 5% probability level ($p < 0.05$). Results were expressed as mean ± standard error of the mean (SEM), and different superscript letters were used in tables to denote statistically significant differences among treatments. All analyses were conducted on tank means (n = 3 per treatment), avoiding pseudoreplication. This design yields ANOVA degrees of freedom of F (3,8) for treatment effects. Graphical representations, including bar plots and boxplots with error bars, were generated using the ggplot2 package in R for clear visualization of treatment effects.

## Results

### *Growth performance*

The growth performance of *M. vittatus* subjected to different dietary levels of ZnONPs over a 75-day feeding period is summarized in Table 1 and visualized in Fig. 1. For all treatments, fish of statistically similar initial weights (0.45±0.001 g) were taken to ensure the homogeneity of experimental fish at the start.

A marked improvement in growth performance was observed with increasing levels of ZnONPs in the diet. The final body weight (FW) increased significantly in a dose-

dependent manner across the treatments. The highest final weight was recorded in fish fed with 110 mg/kg ZnONPs, reaching 1.5±0.001 g, which was significantly greater than other treatments. These results indicate that dietary ZnONPs supplementation enhanced somatic growth in *M. vittatus*.

The weight gain percentage (WG%) showed a similar trend, with $T_3$ achieving the highest gain of 233.33±0.27%, significantly greater than $T_2$ (210.76±0.15%), $T_1$ (189.72±0.92%), and the control (148.57±6.05%). This trend was further reflected in the specific growth rate (SGR%/day), which ranged from 1.21±0.03%/day in $T_0$ to 1.60±0.001%/day in $T_3$, indicating enhanced growth efficiency with increasing levels of ZnONPs.

The feed conversion ratio (FCR) showed a significantly decreasing trend ($p < 0.05$) from 2.11±0.83 in $T_0$ to 1.33±0.001 in $T_3$, suggesting improved feed utilization in fish fed with ZnONPs. Conversely, protein efficiency ratio (PER) increased with ZnONPs supplementation, with $T_3$ exhibiting the highest value (2.01±0.002), indicating better protein assimilation in fish receiving higher doses of ZnONPs. The condition factor (CF) showed no significant variation among the treatments, suggesting that the overall health and well-being of the fish were maintained uniformly across treatments. Survival rate (SR%) was positively influenced by ZnONPs inclusion, increasing from 55.83±0.42% in the control to 67.50±0.73% in $T_3$. Although overall survivability remained relatively low across treatments, likely due to artificial aquarium conditions and the early life-stage sensitivity of this species, ZnONPs supplementation reduced mortality compared to the control, especially in T3. This suggests a potential role of ZnONPs in enhancing the resilience and health of *M. vittatus* under experimental conditions.

### *Hematological metrics*

The major hematological metrics are presented in Table 2. Red blood cell (RBC) counts ranged from 0.48±0.12 $\times 10^6$/µL ($T_2$) to 0.81±0.98 $\times 10^6$/µL ($T_1$), with no significant variation. Hemoglobin (Hb) concentrations remained statistically similar, varying from 3.63±0.47 g/dL to 4.80±0.40 g/dL. White blood cell (WBC) counts, indicative of immune status, showed non-significant differences, ranging from 9.67±3.57 $\times 10^3$/µL to 19.53±3.25 $\times 10^3$/µL. This suggests that dietary ZnONPs did not trigger immune stress or hematological dysfunction. Hematocrit (HCT) values were highest in $T_1$ (7.89±0.92%) and lowest in $T_2$ (4.37±1.12%), but differences were not significant. Plateletcrit (PCT%) remained low and similar across all treatments. Other erythrocytic indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), showed no significant changes. Although numerical variations were observed (e.g., MCHC ranged from 61.29±1.99 g/dL in $T_1$ to 88.73±13.05 g/dL in $T_2$), these were not statistically significant, and did not indicate anemia or hemolytic stress.

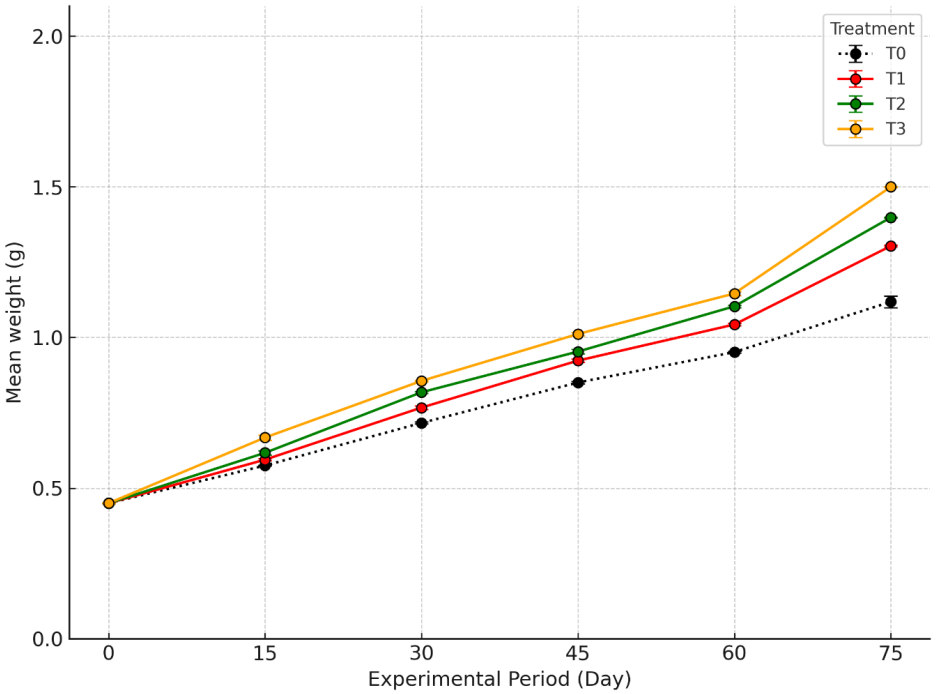


**Fig. 1. Growth curve of *M. vittatus* fed with ZnONPs supplemented diets.**

**Table 1. Growth performance of *M. vittatus* across different treatments (Mean ± SEM)**

| Factors | Treatments | | | |
|---|---|---|---|---|
| | $T_0$ (Control) | $T_1$ (50 mg/kg) | $T_2$ (80 mg/kg) | $T_3$ (110 mg/kg) |
| IW (g) | 0.45±0.001$^a$ | 0.45±0.0$^a$ | 0.45±0.001$^a$ | 0.45±0.001$^a$ |
| FW (g) | 1.119±0.003$^d$ | 1.304±0.00$^c$ | 1.398±0.007$^b$ | 1.5±0.001$^a$ |
| WG (%) | 148.57±6.05$^d$ | 189.72±0.92$^c$ | 210.76±0.15$^b$ | 233.33±0.27$^a$ |
| SGR (%/day) | 1.21±0.03$^d$ | 1.42±0.0004$^c$ | 1.51±0.0006$^b$ | 1.60±0.001$^a$ |
| FCR | 2.11±0.83$^a$ | 1.65±0.0008$^b$ | 1.49±0.001$^c$ | 1.33±0.001$^d$ |
| PER | 1.29±0.52$^d$ | 1.65±0.007$^c$ | 1.83±0.001$^b$ | 2.01±0.002$^a$ |
| CF | 0.65±0.069$^a$ | 0.55±0.014$^a$ | 0.64±0.062$^a$ | 0.60±0.033$^a$ |
| SR% | 55.83±0.42$^c$ | 59.17±0.83$^b$ | 65.00±1.44$^a$ | 67.50±0.73$^a$ |

*Data in the same row bearing different superscript letters differ significantly ($P$<0.05). IW = Initial weight, FW = Final weight, WG% = Weight Gain Percentage, SGR = Specific Growth Rate (%/day), FCR = Feed Conversion Ratio, PER = Protein Efficiency Ratio, CF = Condition Factor, SR% = Survival Rate Percentage.

**Table 2. Hematological parameters of *M. vittatus* under different treatments (Mean ± SEM, n = 3 per treatment)**

| Parameters | Treatments | | | |
|---|---|---|---|---|
| | $T_0$ (Control) | $T_1$ (50 mg/kg) | $T_2$ (80 mg/kg) | $T_3$ (110 mg/kg) |
| RBC ($\times 10^6/\mu L$) | 0.6±0.09 | 0.81±0.10 | 0.48±0.12 | 0.72±0.16 |
| Hb (g/dL) | 4.07±0.38 | 4.8±0.40 | 3.63±0.47 | 4.4±0.51 |
| WBC ($\times 10^3/\mu L$) | 11.7±1.21 | 19.53±3.25 | 9.67±3.57 | 12.83±5.01 |
| HCT (%) | 6.17±1.09 | 7.89±0.92 | 4.37±1.12 | 7.87±1.79 |
| PCT (%) | 0.16±0.03 | 0.20±0.08 | 0.23±0.07 | 0.11±0.04 |
| MCV (fL) | 104±1.90 | 97.34±3.87 | 92.47±4.64 | 109.1±9.61 |
| MCH (pg) | 70.87±7.70 | 59.50±2.55 | 80.43±9.80 | 66.36±14.54 |
| MCHC (g/dL) | 68.9±8.83 | 61.29±1.99 | 88.73±13.05 | 63.13±16.82 |

RBC = Red Blood Cell, Hb = Hemoglobin, WBC = White Blood Cell, HCT = Hematocrit, PCT = Plateletcrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, and MCHC = mean corpuscular hemoglobin concentration.

Hematological indices (WBC, hemoglobin, RBC, and HCT) did not differ significantly among treatments (one-way ANOVA: WBC $F(3,8) = 1.46$, $p = 0.296$; Hb $F = 1.25$, $p = 0.354$; RBC $F = 1.40$, $p = 0.312$; HCT $F = 1.73$, $p = 0.237$; Kruskal–Wallis: all $p > 0.15$). With n = 3 per treatment and noticeable within-group variability, tests had limited statistical power. Effect size analysis showed large variance explained by treatment for HCT ($\eta^2 = 0.394$), WBC (0.354), RBC (0.344), and Hb (0.319). These values suggest biologically meaningful differences among treatments that were not statistically confirmed, likely due to small sample size. In contrast, indices with lower effect sizes reflected little treatment influence, indicating true stability across diets (Supplementary Tables S1). Beyond hypothesis testing, effect sizes with 95% confidence intervals were wide and overlapping, and linear/quadratic trend analyses across ZnONP dose levels were non-significant (Supplementary Tables S2-S5). Numerical values suggested slightly more stable hematological profiles at 80 mg/kg, but these differences were not statistically significant. Overall, ZnONPs at the tested dietary concentrations did not significantly alter hematological health. At the same time, supplementation up to 110 mg/kg diet improved growth, feed utilization, and survival.

## Discussion

Nanoparticles have drawn researchers' attention for their wide applications across various sectors, and their development has opened promising opportunities for advancement in aquaculture (Suvagiya *et al.*, 2024). Today's aquaculture aims to produce more yield within a shorter period. Nanotechnology increases the growth, survival rate, and immunity of the target organisms, thereby supporting this goal (Rakhi *et al.*, 2022). Among multiple uses, the inclusion of nanoparticles in fish diets boosted aquaculture production due to their growth-promoting attribute.

In the present study, supplementation of ZnONPs at 50, 80, and 110 mg/kg was positively correlated with the growth rate. A similar result was reported by Zahran *et al.* (2024), who examined the growth performance of *O. niloticus* fed with algal-sourced zinc nanoparticles. In their study, fish exposed to the highest dose (60 mg/kg) demonstrated the highest growth performance compared to other treatments. In another study on *O. niloticus*, basal diet with 500 mg/kg bacterial zinc nanoparticles yielded the highest growth compared to others (Alsulami and El-Saadony, 2024). Sherif *et al.* (2023) reported that inclusion of ZnONPs at a level of 60 µg/g feed increased phagocytic activity, phagocytic index, serum bactericidal activity, lysozyme, and respiratory burst activity in *O. niloticus*. In a study on broiler chickens, Ahmadi *et al.* (2013) discovered that a diet supplemented with 90 mg/kg of ZnONPs promoted the highest growth, while the control group exhibited significantly lower growth. They proposed that the enhanced growth resulted from the unique physicochemical properties of ZnONPs. Another study compared the effects of bulk zinc oxide and ZnONPs on broiler growth. Similar to the present findings, birds that consumed a ZnONPs-supplemented diet gained significantly more weight than those fed a diet containing bulk zinc oxide (Radi *et al.*, 2021). Badawi *et al.* (2017) reported that Zinc is vital to over 300 enzymes linked with energy, nucleic acid, and protein metabolism. Moreover, ZnONPs can bolster metabolism by triggering insulin and growth hormone gene activity (Ibrahim *et al.*, 2017). Similar to the weight gain pattern, the reduction of FCR and acceleration of PER proceeded with raising ZnONPs. The survival rate percentage (SR%) showed a significant difference, being highest at $T_3$ (67.50 ± 0.73) and lowest at $T_0$ (55.83 ± 0.42). It should be noted, however, that overall survivability across treatments was somewhat low, which can be attributed to the artificial aquarium conditions and the inherent sensitivity of *M. vittatus* during its early life stages (Hossain *et al*., 2006). Additional handling stress during repeated growth sampling may also have contributed to mortality. Nevertheless, the increased SR% was positively correlated with the higher concentrations of ZnONPs, with notably reduced mortality in $T_3$ compared to the control. This trend is consistent with

the findings of Thangapandiyan and Monika (2019), who also reported improved survival with ZnONPs supplementation.

Although the group means of RBC, Hb, WBC, and HCT varied numerically, both parametric and non-parametric tests showed no significant differences among treatments ($p > 0.05$). Similar outcomes have been reported in other nanoparticle studies where hematological indices remained stable. For example, *O. niloticus* exposed to iron-oxide nanoparticles showed no significant changes in hemoglobin, hematocrit, or erythrocyte and leukocyte counts compared to controls (Mohamed *et al.*, 2021). In mammals, administration of Fe-doped ZnO also produced no significant differences in several red cell indices (MCV, MCH, MCHC, RDW) relative to controls (Zhou *et al.*, 2019). Likewise, in a tilapia feeding trial, dietary zinc nanoparticles did not significantly alter hemoglobin concentration (Sivakumar *et al.*, 2020). Although statistical analyses did not confirm significant differences, the 80 mg/kg group showed relatively consistent hematological indices, which may indicate a trend toward physiological stability. This observation, however, requires confirmation with larger sample sizes. On the other hand, studies on common carp have shown significant reductions in RBC and Hb at higher nanoparticle exposures, indicating that hematological responses can vary with species, dose, and exposure conditions (Mumtaz *et al.*, 2021; Khan *et al.*, 2018). Taken together with our present data ($n = 3$ per treatment), the absence of significant treatment effects on RBC, Hb, WBC, and HCT appears biologically reasonable and suggests that moderate levels of dietary ZnONPs do not impair hematological health in *M. vittatus*. Effect size analysis supported this interpretation: large values for RBC, WBC, Hb, and HCT indicate possible biological responses that were not statistically validated, while low effect sizes in other indices suggest true stability across treatments.

The results also demonstrated a statistically significant enhancement in the growth and survival rates of *M. vittatus* across all tested ZnONP doses. The highest dose yielded the greatest improvements in growth performance and survivability, indicating that ZnONPs were non-toxic even at elevated concentrations and can be regarded as an effective feed additive for aquaculture applications.

## Conclusions

The experimental results indicate that dietary supplementation of zinc oxide nanoparticles at 110 mg/kg produced the highest growth performance and survival rate in *M. vittatus*, making this dose most beneficial from a production standpoint. Hematological analysis, however, showed that fish at 80 mg/kg had numerically more stable blood indices (RBC, WBC, Hb, HCT), although these differences were not statistically significant. This suggests that while 110 mg/kg maximized growth and survival, 80 mg/kg may represent a physiologically stable option. It should also be acknowledged that overall mortality was somewhat higher than expected,

mainly due to artificial rearing conditions and species-specific vulnerability at early life stages. Thus, the optimal level may depend on production goals: 110 mg/kg for short-term growth and yield, and 80 mg/kg for long-term health and sustainability. Overall, the study demonstrates that ZnONPs enhanced growth and survivability in *M. vittatus* without evidence of adverse hematological effects.

## Supplementary Materials

Supplementary Tables S1-S5

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### *Conflict of Interest*

The authors affirm that no financial or commercial relationships that might be construed as a potential conflict of interest existed during the course of the research.

### *Author Contributions*

Methodology, Formal Analysis, and Writing – Original Draft: Md. Mahadi Hasan. Review and Editing: Md. Shah Newaz, Md. Arif Shahariar, Md. Zunaed Hossain and Riad Ahmed. Conceptualization, Methodology, Analysis, Project Administration, Review and Editing, and Funding Acquisition: Mohammad Shafiqul Alam

### *Ethics approval and consent to participants*

The Institutional Animal Research Ethics Committee (AREC) of the Gazipur Agricultural University declares that the experiments were carried out according to the committee guidelines, and performed with their knowledge and permission (Ref. No. FVMAS/AREC/2023/31).

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