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Length-Weight Relationship of Labeo rohita Fingerlings Fed Diets Supplemented with Commercial Zinc Oxide Nanoparticles

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Abstract
The length-weight relationship (LWR) is an important biological tool for assessing fish growth patterns and evaluating the influence of nutritional interventions in aquaculture. This study investigated the effect of dietary supplementation with commercial zinc oxide nanoparticles (C-ZnO NPs) on the length-weight relationship of Labeo rohita fingerlings. Healthy fingerlings (initial mean body weight: 2.23±0.38 g; total length: 5.74±0.28 cm) were randomly assigned to four dietary treatments: a basal diet (control) and basal diets supplemented with commercial ZnO nanoparticles at 10, 100, and 200 mg/kg of feed. Fish were reared in triplicate for 90 days under controlled laboratory conditions, and water quality parameters were maintained within the recommended range throughout the experiment. The relationship between body length and weight was evaluated using regression analysis. A strong positive association between body length and weight was observed in all dietary treatments, with coefficients of determination (R^2) ranging from 0.7824 to 0.8794. The control group exhibited the highest coefficient of determination (R^2=0.8794), while the nanoparticle-treated groups recorded R^2 values of 0.7942, 0.7824, and 0.8253 for the 10, 100, and 200 mg/kg treatments, respectively. These findings indicate that dietary supplementation with commercial ZnO nanoparticles maintained a consistent relationship between body length and weight without markedly altering the overall growth pattern of L. rohita fingerlings under the experimental conditions. The present study provides baseline information on the length-weight relationship of L. rohita following dietary supplementation with commercial ZnO nanoparticles and contributes to the understanding of nanoparticle-based mineral nutrition in aquaculture.

Keywords: Labeo rohita; commercial zinc oxide nanoparticles; length-weight relationship; aquaculture; fish growth; regression analysis.

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Introduction
Length-weight relationship (LWR) is one of the most widely used biological parameters in fish biology and fisheries science. It provides a simple and reliable method for estimating fish weight from length measurements or vice versa and has broad applications in fisheries assessment, stock evaluation, and aquaculture research. Besides estimating biomass, LWR is widely used to assess fish condition, compare populations from different habitats, and evaluate growth patterns under varying environmental and nutritional conditions. The allometric coefficient (b) derived from the LWR indicates whether fish exhibit isometric or allometric growth and provides valuable information on stock composition, growth performance, mortality, production, and population dynamics (Goncalves et. al., 1996; Kashyap et. al., 2014; Le Cren, 1951; Yousaf et al., 2009). Thus, LWR has become an indispensable tool for understanding fish growth and assessing the effectiveness of nutritional and management interventions in aquaculture. Among the nutritional factors influencing fish growth, zinc (Zn) is an essential trace element required for numerous physiological and biochemical processes. It functions as a cofactor for a wide range of enzymes involved in protein and nucleic acid synthesis, carbohydrate metabolism, antioxidant defence, tissue repair, and immune regulation (Huang et al., 2015; Kumar et al., 2018). Adequate zinc nutrition is therefore essential for maintaining normal growth, skeletal development, appetite regulation, stress tolerance, and disease resistance in cultured fish (Rajan and Rohini, 2021; Rashidian et al., 2021). Although freshwater fish can absorb zinc from both water and dietary sources, the concentration of dissolved zinc in freshwater is generally insufficient to meet their physiological requirements. Furthermore, the bioavailability of dietary zinc is often reduced by interactions with phytates and competing minerals such as calcium and phosphorus present in feed ingredients, making dietary zinc supplementation necessary in aquaculture (Watanabe et al., 1997). Traditionally, dietary zinc has been supplied through inorganic salts such as zinc sulfate ($ZnSO_4$), zinc oxide (ZnO), and zinc chloride, while organic sources including zinc amino acid chelates and zinc-enriched yeast have also been developed to improve absorption (Padmavathy and Vijayaraghavan, 2008; Shah and Mraz, 2020). Although inorganic zinc sources are inexpensive and widely available, they generally exhibit low bioavailability because a considerable proportion of the supplemented mineral forms insoluble complexes within the digestive tract and is subsequently excreted. Excessive supplementation is therefore often required to satisfy the nutritional needs of fish, resulting in reduced nutrient utilization and increased discharge of unutilized zinc into aquatic ecosystems (Apines et al., 2001; Agarwal et al., 2017). Such mineral losses not only decrease feed efficiency but may also contribute to

environmental contamination and ecological imbalance (Jarvie et al., 2009; Kumar et al., 2018). These limitations have encouraged the search for alternative zinc sources that combine high bioavailability with improved environmental sustainability. Recent advances in nanotechnology have opened new opportunities for improving nutrient delivery in aquaculture. Nanomaterials possess unique physicochemical characteristics, including nanoscale particle size, large surface area, enhanced reactivity, improved cellular uptake, greater digestive stability, and controlled nutrient release, making them attractive alternatives to conventional mineral supplements (Rather et al., 2011; Marappan Gopi et al., 2017; Jewel et al., 2023). Various nano minerals, including zinc oxide (ZnO), selenium, iron oxide, chromium, copper oxide, magnesium oxide, and titanium dioxide nanoparticles, have been investigated as dietary supplements because of their potential to enhance nutrient utilization, improve feed efficiency, strengthen immune responses, and promote growth in cultured fish (Khosravi-Katuli et al., 2017; Swain et al., 2019; Tabassum et al., 2020). The reduced supplementation requirement and lower nutrient losses associated with nano minerals may also help minimize environmental pollution resulting from conventional mineral additives. Among these nano minerals, zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention as an alternative dietary zinc source. Owing to their small particle size and high surface-area-to-volume ratio, ZnO nanoparticles exhibit superior solubility, intestinal absorption, and bioavailability compared with conventional zinc supplements (Agarwal et al., 2017; Kumar et al., 2018). Therefore, evaluating both the benefits and potential risks of dietary ZnO nanoparticles is essential before their large-scale adoption in aquaculture. Although numerous studies have investigated the effects of ZnO nanoparticles on growth performance, antioxidant status, and immune responses, information regarding their influence on the length-weight relationship and growth pattern of cultured fish remains limited.

Therefore, the present study was undertaken to evaluate the length-weight relationship of *L. rohita* fed diets supplemented with commercial zinc oxide nanoparticles. The findings are expected to provide insights into the effects of dietary ZnO nanoparticles on fish growth characteristics and contribute to the development of efficient and sustainable zinc supplementation strategies for aquaculture.

Materials and Methods

1. Experimental fish and acclimatization

Healthy fingerlings of *L. rohita* with an initial mean body weight of 2.23±0.38 g and total length of 5.74±0.28 cm were procured from a commercial hatchery located in Medchal, Hyderabad, Telangana, India. The fish were transported to the Wet Laboratory of the Department of Zoology, Maulana Azad National Urdu University (MANUU), Hyderabad, in aerated plastic containers to minimize transport stress. Upon arrival, the fingerlings were transferred to dechlorinated freshwater tanks and acclimatized for two weeks under laboratory conditions with continuous aeration. During acclimation, the fish were fed a commercial basal diet twice daily, and water quality was maintained within the optimum range for *L. rohita*. Only healthy and active fish were selected for the feeding experiment.

2. Commercial zinc oxide nanoparticles

Commercial zinc oxide nanoparticles (C-ZnO NPs) with an average crystallite size of approximately 71 nm were procured from Sigma-Aldrich, India, and used as the dietary zinc source throughout the experiment.

3. Experimental design

A 90-day feeding trial was conducted to evaluate the effect of dietary commercial zinc oxide nanoparticles on the length-weight relationship of *L. rohita*. Four dietary treatments were established: a control diet without ZnO nanoparticles and three experimental diets supplemented with commercial ZnO nanoparticles at 10, 100, and 200 mg/kg feed. Each treatment was maintained in triplicate using glass aquaria (90 × 30 × 35 cm), resulting in a total of 12 experimental tanks. Each aquarium contained 20 fingerlings of uniform size and was maintained with 60 L of dechlorinated freshwater under continuous aeration. Fish were randomly distributed among the tanks to minimize experimental bias. All experimental groups were maintained under identical environmental and husbandry conditions throughout the feeding trial.

4. Preparation of experimental diets

A commercial basal diet containing 30% crude protein (Hi-Pro Feeds India Pvt. Ltd., Hyderabad, India) served as the control diet and as the base for nanoparticle supplementation. Commercial ZnO nanoparticles were incorporated into the basal diet at concentrations of 10, 100, and 200 mg/kg following a modified protocol of Muralisankar et al. (2014). Details of the diet preparation procedure have been described previously by Rasool et al. (2024).

5. Water quality

Water quality was monitored regularly throughout the 90-day feeding trial using a NICE® water quality testing kit (India). Water temperature and pH were recorded routinely, whereas dissolved oxygen (DO), ammonia (NH_3), nitrate (NO_3^-), and nitrite (NO_2^-) concentrations were measured twice weekly. Throughout the experimental period, the physicochemical parameters remained within the acceptable range for *L. rohita* culture. The mean (± SD) values recorded were DO 8.08 ± 0.55 mg/L, water temperature 29.02 ±

0.91°C, ammonia 0.68 ± 0.62 mg/L, nitrate 3.42 ± 2.30 mg/L, and nitrite 0.45 ± 0.14 mg/L, while the pH ranged between 6.5 and 7.5. No noticeable differences were observed among the control and ZnO nanoparticle-treated groups, indicating that dietary supplementation did not adversely affect water quality. Maintaining these parameters within the optimal range minimized environmental stress and ensured that any observed differences in growth characteristics were attributable to the dietary treatments rather than water quality fluctuations.

6. *Length-Weight Relationship*

The Length-Weight Relationship (LWR) was analysed using the classical equation proposed by Le Cren (1951): W=aLb
where *W* is total body weight (g), *L* denotes total length (cm), *a* is intercept, and *b* is allometric growth exponent. The equation was linearized by logarithmic transformation to facilitate regression analysis: logW=loga+blogL

Linear regression was applied to estimate the parameters a and b using least squares method. The coefficient of determination (R^2) was calculated to assess the fit of the model. According to Le Cren (1951), the growth of the fish is considered isometric when b=3, positive when b is greater 3, while a value lower than three is a metric of negative allometric growth.

7. *Statistical analysis*

The length-weight relationship was analysed using linear regression after logarithmic transformation of the data. Regression parameters, including the intercept (a), allometric growth coefficient (b), and coefficient of determination (R^2), were estimated using the least-squares method. All statistical analyses and graphical representations were performed using Microsoft Excel 2019.

Results

1. *Procurement and characterization of commercial zinc oxide nanoparticles*

Commercial zinc oxide nanoparticles (C-ZnO NPs) with an average crystallite size of approximately 71 nm were procured from Sigma-Aldrich, India. The nanoparticles were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM) to verify their crystalline structure and surface morphology prior to dietary application. XRD analysis confirmed the characteristic hexagonal wurtzite crystalline structure of ZnO nanoparticles, while SEM micrographs revealed predominantly spherical particles with moderate agglomeration. The representative XRD pattern and SEM images are presented in Figure 1a and 1b, confirming the structural integrity and morphology of the commercial ZnO nanoparticles used in the feeding trial.

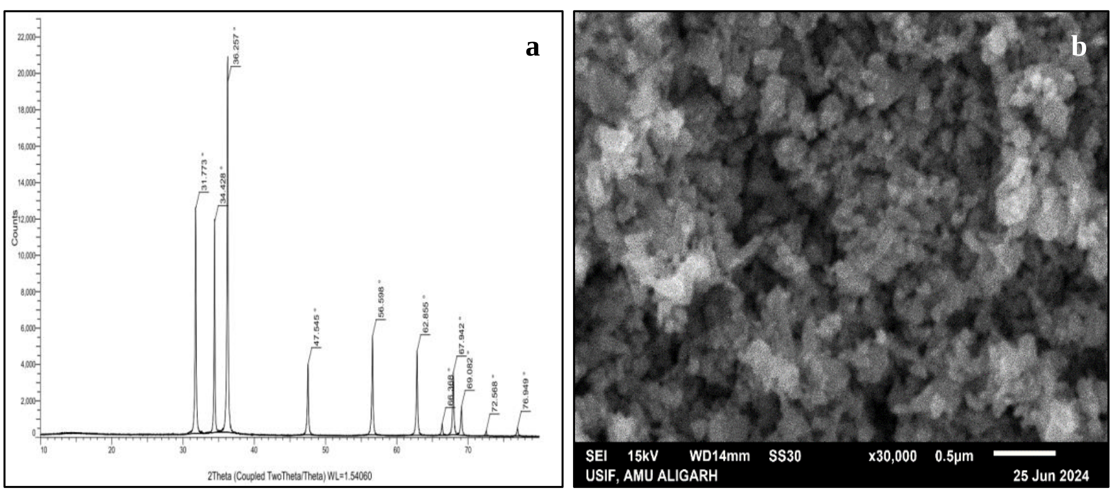

Figure 1: Characterization of C-ZnO NPs: XRD spectra (a) and scanning electron microscope image (b).

2. *Relationship between length and weight*

The relationship between body length and weight of *L. rohita* fingerlings fed diets supplemented with commercial zinc oxide nanoparticles (C-ZnO NPs) is presented in Figures 2 and 3. A positive linear relationship was observed between body weight and total length in all dietary treatments, indicating that fish increased in length with increasing body weight throughout the experimental period. The control group (basal diet) exhibited the strongest relationship between length and weight, with the regression equation y=2.5559x-1.6265 and a coefficient of determination (R^2=0.8794) (Figure 2). Fish fed the diet supplemented with 10 mg/kg C-ZnO showed a regression equation of y=2.1056x-1.1989 with an R^2 value of 0.7942 (Figure 3a). Similarly, the 100 mg/kg C-ZnO group produced the regression equation y=2.1700x-1.2322 with an R^2 value of 0.7824 (Figure 3b), whereas the 200 mg/kg C-ZnO group exhibited the equation y=1.9464x-1.0519 with an R^2 value of 0.8253 (Figure 3c).

FishTaxa - Journal of Fish Taxonomy
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The coefficients of determination ranged from 0.7824 to 0.8794, demonstrating a strong positive association between body length and weight in all experimental groups. Among the nanoparticle-treated groups, the 200 mg/kg treatment showed the highest R^2 value (0.8253), indicating a relatively stronger linear association than the 10 and 100 mg kg^{-1} treatments, although it remained slightly lower than that of the control group. Overall, dietary supplementation with commercial ZnO nanoparticles maintained a consistent positive relationship between body length and weight without marked changes in the growth trend of *L. rohita* fingerlings.

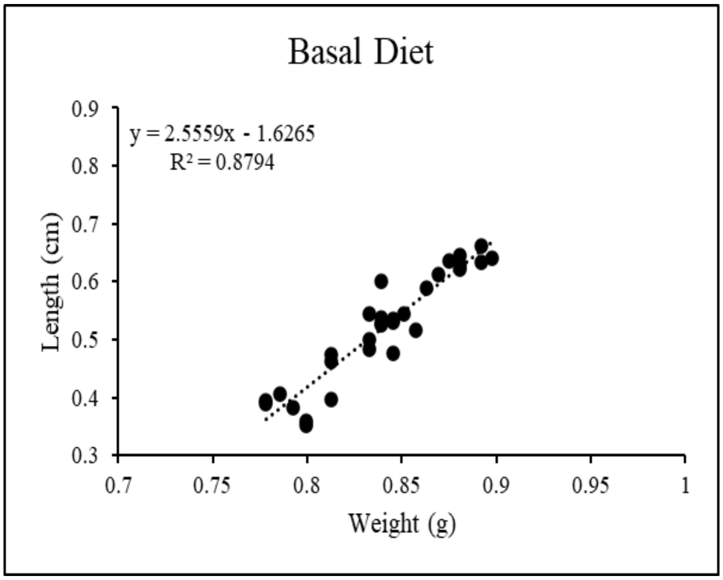

Figure 2: Linear relationship between total length and body weight of *L. rohita* fingerlings (n=10) fed the basal diet.

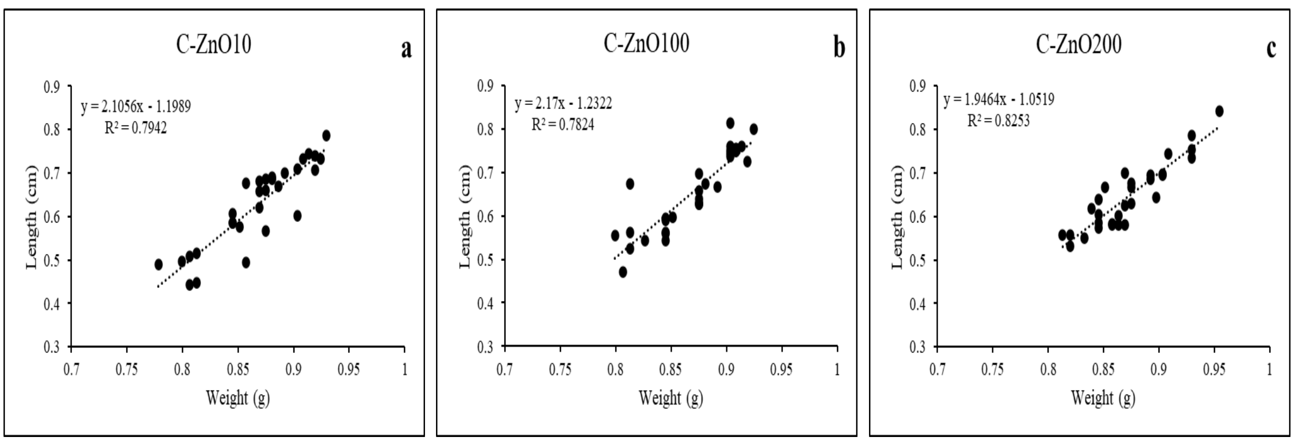

Figure 3: Linear relationship between total length and body weight of *L. rohita* fingerlings (n=10) fed diets supplemented with commercial zinc oxide (C-ZnO) nanoparticles at different concentrations.

Discussion

The length-weight relationship (LWR) is a fundamental tool in fisheries biology and aquaculture for describing growth patterns and assessing the condition of fish populations. The relationship between body weight (W) and total length (L) is commonly expressed by the equation $W=aL^b$, where the allometric coefficient (*b*) provides information on growth characteristics (Le Cren, 1951). LWR has broad applications in biomass estimation, stock assessment, feed management, and evaluation of fish health and environmental adaptation (Froese, 2006; Jana et al., 2022). According to Froese (2006), variations in the *b* value are influenced by several biological and environmental factors, including age, sex, nutritional status, food availability, habitat, and water quality.

In the present study, *L. rohita* fingerlings exhibited negative allometric growth ($b<3$) in all dietary treatments, indicating that body length increased at a relatively faster rate than body weight during the experimental period. This growth pattern is frequently reported in juvenile fishes, where skeletal elongation precedes substantial muscle deposition and body mass accumulation (Froese, 2006). The control group also exhibited negative allometric growth, suggesting that this growth pattern was characteristic of the developmental stage of the experimental fish rather than solely a consequence of dietary supplementation. Similar observations have been reported in carp fingerlings by Masram et al. (2022), who described negative allometric growth during the juvenile phase. Dietary supplementation with commercial ZnO nanoparticles did not alter the overall growth pattern of *L. rohita*, as all nanoparticle-treated groups also exhibited negative allometric growth. However, slight differences in the estimated *b* values among treatments suggest that dietary ZnO nanoparticles may influence the proportional relationship between body length and weight. Zinc is an essential trace mineral involved in numerous enzymatic reactions associated with protein synthesis, antioxidant defence, cellular metabolism, and immune regulation (Kumar et al., 2018; Rajan and Rohini, 2021). The improved bioavailability of ZnO nanoparticles compared with conventional zinc supplements has been associated with enhanced nutrient utilization and growth performance in several fish species (Swain et al., 2019; Rashidian et al., 2021). Nevertheless, under the present experimental conditions, dietary supplementation with commercial ZnO nanoparticles did not induce a shift from negative allometric to isometric growth. A gradual reduction in the estimated *b* value with increasing ZnO nanoparticle supplementation was observed, particularly at the highest dietary concentration. Although the biological mechanism underlying this trend was not investigated in the present study, it may indicate that increasing dietary ZnO nanoparticle levels did not proportionally enhance body weight gain relative to length growth. Similar observations have been reported in studies where excessive mineral supplementation reduced growth performance or feed utilization efficiency in freshwater fishes (Getso et al., 2017; Sheikh and Ahmed, 2018).

The present findings demonstrate that dietary supplementation with commercial ZnO nanoparticles maintained a normal juvenile growth pattern in *L. rohita* fingerlings while preserving a strong relationship between body length and weight. These results provide baseline information on the influence of dietary ZnO nanoparticles on fish growth characteristics and contribute to the understanding of nanoparticle-based mineral supplementation in aquaculture.

Conclusion

The present study evaluated the influence of dietary supplementation with C-ZnO NPs on the length-weight relationship of *L. rohita* fingerlings. A strong positive relationship between body length and weight was observed in all dietary treatments, with high coefficients of determination (R^2=0.7824-0.8794), indicating a consistent association between these growth parameters. All experimental groups exhibited a negative allometric growth pattern, suggesting that body length increased at a faster rate than body weight during the juvenile stage. Although slight variations in the regression parameters were observed among the ZnO nanoparticle-supplemented groups, dietary C-ZnO nanoparticles did not substantially alter the overall growth pattern of the fish. These findings indicate that dietary supplementation with commercial ZnO nanoparticles maintained normal growth characteristics of *L. rohita* fingerlings under the experimental conditions. The study provides baseline information on the length-weight relationship of ZnO nanoparticle-fed *L. rohita* and may serve as a useful reference for future investigations on nanoparticle-based mineral nutrition in aquaculture.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Author Contributions

Fazle Rasool: Experimental work, methodology, investigation, data curation, formal analysis, visualization, writing-original draft preparation, and literature review. Masroor Fatima: Conceptualization, supervision, project administration, validation, writing review and editing. Both the authors have read and approved the final version of the manuscript.

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