

DIETARY SILICA NANOPARTICLE AMELIORATES THE GROWTH PERFORMANCE AND MUSCLE COMPOSITION OF STINGING CATFISH, *Heteropneustes fossilis*

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ABSTRACT:

Silica nanoparticles (Si-NPs) are increasingly prevalent in various industrial applications, potentially impacting aquaculture. The study investigated the effects of dietary Si-NPs on growth performance and its repercussions on muscle compositions in Stinging catfish, *Heteropneustes fossilis* (*H. fossilis*). In this study, four isonitrogenous diets containing Si-NPs (0, 1, 2, and 3 mg/kg) were fed to juvenile *H. fossilis* for 60 days. Several growth indices (weight gain, WG; percent weight gain, %WG; length gain, LG; and specific growth rate, SGR), feed utility parameters (feed conversion ratio, FCR; feed conversion efficiency, FCE; and protein efficiency ratio, PER) and survival rate was assessed at the end of the feeding trial. This study showed significant effects of Si-NPs (2 mg/kg) in the growth and muscle composition of *H. fossilis*. However, Si-NPs did not significantly affect the feed utility of *H. fossilis*. The findings of this study recommended that Si-NPs can be effectively supplemented into the diets of *H. fossilis* for better production.

KEYWORDS: Silica nanoparticle; feed; growth; muscle composition; Stinging catfish.

1. INTRODUCTION

Aquaculture is one of the fastest-growing and most influential animal protein sectors globally (Naylor *et al.*, 2021; Hoseini & Al Sulivany, 2024). Particularly for developing Asian and African nations like Bangladesh, this expanded industry has been shown to contribute significantly to food security (Chan *et al.*, 2019). Among the inputs for aquaculture feed accounts for nearly 60-70% of the total production costs which quality must be met to ensure a sustainable production (Akter *et al.*, 2021). Quality feed is the prerequisite for successful aquaculture operations, and it significantly influences the production and profitability of this sector (Singha *et al.*, 2021; Al Habbib & Al Sulivany, 2013). Nowadays, the sustainability of aquaculture is a great challenge due to the ceiling price of the feed ingredients that reduce the profitability of this emerging sector (Daniel *et al.*, 2018). To overcome this significant problem, nutritionists and aquaculturists from all over the world have introduced several strategies such as feed additives, including probiotics (Rohani *et al.*, 2022a; Islam *et al.*, 2021; Jahan *et al.*, 2021); prebiotics (Rohani *et al.*, 2019a; Islam *et al.*, 2020); seaweed (Siddik *et al.*, 2023); micronutrients (Rohani *et al.*, 2019b; Rohani *et al.*, 2022b; Rohani *et al.*, 2023) and nanoparticles (Ghafarifarsani *et al.*, 2024).

Nanoparticles (NPs) are a promising supplement in fish feed, offering a range of benefits for aquaculture. (Sarkar *et al.*, 2022). They have been studied for their potential to enhance the bioavailability and absorption of nutrients, thereby playing a significant role in improving the growth and health of farmed fish species. (Misra *et al.*, 2023). By increasing the bioactivity of molecules, particularly micronutrients, NPs can improve aquaculture productivity (Shah & Mraz, 2020). Additionally, it

can allow for the tissue-specific application of disease treatments without compromising human health (Jennings *et al.*, 2016). Because of their large specific surface area, NPs can be used as feed additives because they help both terrestrial and aquatic animals absorb micronutrients from the intestine into the bloodstream (Khosravi-Katuli *et al.*, 2017; Pieszka *et al.*, 2019). Application of Silica nanoparticles (Si-NPs) in the production of animals, including fish, is gaining popularity due to their exceptional optical properties, adsorption capacity, low toxicity, biocompatibility, thermal stability, and low production cost (Bitar *et al.*, 2012; Priyadarsini *et al.*, 2018). It has been reported that Si-NPs improve fish welfare by making medication administration easier and lowering the chance of disease outbreaks, even in cases of extreme crowding (Khosravi-Katuli *et al.*, 2017). Furthermore, studies have shown that Si-NPs are helpful for treating wastewater (Jarvie *et al.*, 2009), managing the microbial load (Huang *et al.*, 2015), and promoting the growth of aquatic species (Bashar *et al.*, 2021).

Stinging catfish (*Heteropneustes fossilis*) along with other catfish species, play a significant role in global aquaculture (Ali *et al.*, 2018). This species is considered a good source of protein with some medicinal benefits (Rahman *et al.*, 2014). With excellent suitability in different aquaculture systems (Mahmud *et al.*, 2017), tanks (Ahamed *et al.*, 2023), and recirculatory and bio-flock systems (Sohel *et al.*, 2023) the importance of this species has increased. Despite the remarkable potential of Si-NPs in aquaculture, there is very limited information regarding their role in important farmed species, such as *H. fossilis*. Therefore, the current study was carried out to investigate the effects of dietary Si-NPs on growth performance, feed utilization, and muscle composition in *H. fossilis*.

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2. MATERIALS AND METHODS

Ethical Issues:

The Bangladesh Agricultural University Research System's ethical committee reviewed and approved the experimental design and implementation (Code Number: BAU-FoF/2023/005). The fish were fed, handled, and sacrificed according to the animal welfare and ethical committee's established regulations.

Experimental Fish:

Stinging catfish fry, weighing 1.00 g (\pm 0.10 g) from the same breeding stock, were procured from Reliance Hatchery Ltd., Mymensingh, Bangladesh. There was no sign of diseases and/or abnormalities. After collection, the fry was acclimatized for 7 days in the experimental system. Fish were fed a controlled diet, and an oxygen supply was kept consistent during the acclimation period. After 7 days, fish were randomly assigned to the replication tanks at a density of 94 per cubic meter (15 fish per 160 L tank).

Physicochemical Parameters:

The water quality parameters in the aquaculture tanks were evaluated using various instruments and a multimeter. These parameters included temperature (24.3°C), pH (6.3), electrical conductivity (453 μ S/cm), total dissolved solids (290 ppm), turbidity (3.4 NTU), dissolved oxygen (5.9 mg/L), total hardness (238 mg/L), 5-day biological oxygen demand (2.7 mg/L), and total alkalinity (135 mg/L).

Experimental Design:

Four diets with graded Si-NPs (0, 1, 2 and 3 mg/kg) were fed to *H. fossilis*. A static aquaria system with 20 rectangular glass tanks (0.64m \times 0.5m \times 0.5m) was developed in the Wet Laboratory, Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Five replications for each dietary treatment were used. Fifteen fry of uniform body weight (1.00 \pm 0.10 g) and length (5.59 \pm 0.14 cm) were

randomly stocked in each tank. The fish were fed for 60 days. Twenty-four hours of continuous aeration and a water depth of 0.4m were maintained throughout the feeding trial. The overview of the experimental design is presented in the following figure:

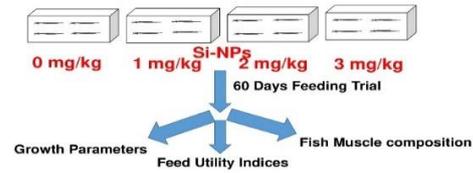


Figure: Overview of experimental design

Diet Formulation and Feeding Trial

Highly pure Si-NPs composed of more than 98% of silicon dioxide (SiO₂), 0.08% Al₂O₃, 0.05% Fe₂O₃, and CaO, 0.5% K₂O, and 0.10% TiO₂, were imported from Canada and activated in Fish Nutrition Laboratory of Department of Aquaculture, BAU. Feed was formulated with locally available ingredients, including fish meal, mustard oil cake, soybean meal, rice bran, wheat bran, molasses, vitamins and minerals premix, etc. Protein level was maintained at 30% for the experimental fishes. Four different dosages of Si-NPs were added to diets, namely 0 mg/kg (T₀, control), 1 mg/kg (T₁), 2 mg/kg (T₂), and 3 mg/kg (T₃). The proximate composition analysis of the formulated diets (Table 1) was carried out in the Fish Nutrition Laboratory, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, by following standard procedures (AOAC, 2005). Pellet feed was made with the help of a pelletizer and kept in air-tight poly bags at -20°C until further use, following a 4-day drying period. The diets were provided at 5% of the body weight, having two times feeding frequency (9 am and 5 pm) for 60 days. Fish were sampled bi-weekly to monitor the growth and adjust the daily ration

Table 1: The inclusion level of Si-NPs and proximate composition of the formulated diets

Name of Items	T ₀	T ₁	T ₂	T ₃
Silica NP (mg)	0	1	2	3
Moisture (%)	13.85	13.3	12.91	13.16
Crude Lipid (%)	5.89	6.24	6.35	5.95
Crude Protein (%)	30.04	30.11	30.18	30.01
Ash (%)	8.27	9.2	9.23	9.55
Crude Fiber (%)	5.23	5.47	5.15	5.21
Carbohydrate (%)	35.86	34.22	33.68	33.17

Growth and Feed Utilization Parameters:

At the end of the feeding trial, growth indices were calculated according to the following formulae (Kari *et al.*, 2023; Al Sulivany *et al.*, 2024):

1. Weight gain (g) = Final weight (g) – Initial weight(g)
2. Percent weight gain (%) = (Weight gain (g)/ (Initial weight (g)) \times 100
3. Specific growth rate (% per day) = (Ln (final weight) – Ln (initial weight))/ (study period (day)) \times 100.
4. Daily growth coefficient (% per day) = (final weight (g)^{0.33} – initial weight(g)^{0.33})/ (study period (day)) \times 100
5. Condition factor = (final weight (g)/ (final length (cm)³)
6. Survival rate (%) = (final number)/ (initial number) \times 100

Feed utilization parameters were calculated from the following formulae (Roslan *et al.*, 2024):

7. Food conversion ratio = (dry feed fed (kg))/ (live weight gain (kg))
8. Food efficiency ratio = (live weight gain (kg))/ (dry feed fed (kg))
9. Protein efficiency ratio = (total weight gain (g))/ (protein intake (g))

Fish Muscle Composition:

At the end of the feeding trial, five fish from each replication were caught by a dip net, killed by a sharp blow on the head, and dissected by scissors to determine the proximate composition of the muscle (Adineh *et al.*, 2024). The contents of crude protein, crude lipid, moisture, and ash were calculated following AOAC (2005). The total nitrogen content was determined using the micro-Kjeldahl analysis (method 945.01) and multiplied by the conversion factor (6.25) to translate it into the total crude protein content. Crude fat, ash, and moisture content were estimated by Soxhlet extraction (method 920.39C), by calcination in a muffle furnace at 550°C for 5 h (method 942.05), and by drying in a hot-air oven at 105°C (method 950.01), respectively. The crude fiber

content (only for feed samples) was estimated with Fiber Tech (Tulin equipment, India) following the calcination in a muffle furnace.

Statistical Analysis:

The data were analyzed using R-Studio (RStudio Team, 2022) and presented as mean \pm SD. The significance of various levels of Si-NPs on the measured responses was ascertained using a one-way ANOVA. To specify the differences among the treatments, a multiple-range test of Tukey at a 5% significance level was performed. $p < 0.05$ was considered to be statistically significant.

3. RESULTS

Growth Performance and Feed Efficiency:

This study evaluated the effects of different level of dietary Si-NPs on growth parameters. The results showed significant differences between the groups in various growth metrics (Table 2). For final weight, significant differences were observed, with T₂ achieving the highest value (8.88 \pm 0.481 g), significantly higher than T₀ (6.85 \pm 0.248 g) ($p < 0.01$). T₃ and T₁ had final weight of 8.29 \pm 0.6 g and 7.35 \pm 0.342 g, respectively, indicating dose-dependent effects. The final length also followed a similar pattern, with T₂ having the longest length (12.19 \pm 0.352 cm), significantly greater than T₀ (10.77 \pm 0.208 cm) ($p < 0.01$). T₃ and T₁ showed intermediate increases (11.68 \pm 0.307 cm and 11.27 \pm 0.271 cm, respectively).

Survival rate, T₂ had the highest rate (93.33%), followed by T₀ (90%) and T₁ (80%), while T₃ showed a significant drop

(70%) ($p < 0.01$). In terms of weight gain, T₂ demonstrated the most substantial increase (7.6 \pm 0.495 g), significantly greater than T₀ (5.68 \pm 0.255 g) ($p < 0.01$). T₃ (7.03 \pm 0.596 g) and T₁ (6.12 \pm 0.339 g) also showed marked improvements. The SGR was significantly higher in T₂ (3.22 \pm 0.108%), followed by T₃ (3.13 \pm 0.119%) and T₁ (2.98 \pm 0.077%), with the control showing the lowest value (2.95 \pm 0.077%) ($p < 0.05$). Daily weight gain mirrored these trends, with T₂ having the highest value (0.13 \pm 0.008 g), significantly greater than T₀ (0.09 \pm 0.004 g) ($p < 0.01$). T₃ and T₁ had daily weight gain of 0.12 \pm 0.01 g and 0.1 \pm 0.006 g, respectively. On the other hand, the condition factor in the control (0.55 \pm 0.016) had the highest value, with a significant decrease in T₁ (0.51 \pm 0.018) and T₂ (0.49 \pm 0.023) ($p < 0.01$), although T₃ showed a slight recovery (0.52 \pm 0.01).

Furthermore, the percent weight gain was the highest value in T₂ (593.47 \pm 45.796%), followed by T₃ (555 \pm 45.611%), with T₀ showing the lowest value (489.06 \pm 27.346%) ($p < 0.05$). T₁ exhibited a moderate improvement (497.33 \pm 27.323%).

The food conversion ratio for T₀ averaged 1.94 \pm 0.065, while the values for T₁, T₂, and T₃ were 2.03 \pm 0.082, 2.05 \pm 0.134, and 2.03 \pm 0.126, respectively, with no statistically significant differences observed ($p = 0.3999$). Similarly, the food conversion efficiency showed no substantial variation between groups, with T₀ recording a mean of 0.52 \pm 0.017, and T₁, T₂, and T₃ yielding 0.49 \pm 0.02, 0.49 \pm 0.033, and 0.49 \pm 0.029, respectively ($p = 0.3902$). Likewise, protein conversion efficiency remained consistent across treatments, with T₀ displaying a mean of 1.72 \pm 0.057 and T₁, T₂, and T₃ showing means of 1.64 \pm 0.066, 1.63 \pm 0.109, and 1.65 \pm 0.096, respectively ($p = 0.3902$).

Table 2: The effects of Si-NPs on growth performance and feed efficiency of *H. fossilis* after 60 days of feeding trial

Growth parameters	Control (T ₀)	T ₁	T ₂	T ₃	P-values
Initial Weight (g)	1.16 \pm 0.027	1.16 \pm 0.014	1.16 \pm 0.015	1.16 \pm 0.014	0.3116
Initial Length (cm)	5.42 \pm 0.104	5.43 \pm 0.096	5.42 \pm 0.117	5.44 \pm 0.115	0.3212
Final Weight (g)	6.85 \pm 0.248 ^b	7.35 \pm 0.342 ^b	8.88 \pm 0.481 ^a	8.29 \pm 0.6 ^a	<0.01
Final Length (cm)	10.77 \pm 0.208 ^c	11.27 \pm 0.271 ^{bc}	12.19 \pm 0.352 ^a	11.68 \pm 0.307 ^{ab}	<0.01
Survival Rate (%)	90 ^b	80 ^c	93.33 ^a	70 ^d	<0.01
Weight Gain (g)	5.68 \pm 0.255 ^b	6.12 \pm 0.339 ^b	7.6 \pm 0.495 ^a	7.03 \pm 0.596 ^a	<0.01
Length Gain (cm)	5.35 \pm 0.187 ^c	5.66 \pm 0.282 ^{bc}	6.39 \pm 0.329 ^a	5.96 \pm 0.307 ^{ab}	<0.01
Specific growth rate (%)	2.95 \pm 0.077 ^b	2.98 \pm 0.077 ^b	3.22 \pm 0.108 ^a	3.13 \pm 0.119 ^{ab}	<0.05
Daily weight gain (g)	0.09 \pm 0.004 ^b	0.1 \pm 0.006 ^b	0.13 \pm 0.008 ^a	0.12 \pm 0.01 ^a	<0.01
Condition factor	0.55 \pm 0.016 ^a	0.51 \pm 0.018 ^b	0.49 \pm 0.023 ^b	0.52 \pm 0.01 ^{ab}	<0.01
Percent weight gain	489.06 \pm 27.346 ^b	497.33 \pm 27.323 ^b	593.47 \pm 45.796 ^a	555 \pm 45.611 ^{ab}	<0.05
Feed parameters					
Food conversion ratio	1.94 \pm 0.065	2.03 \pm 0.082	2.05 \pm 0.134	2.03 \pm 0.126	0.3999
Food conversion efficiency	0.52 \pm 0.017	0.49 \pm 0.02	0.49 \pm 0.033	0.49 \pm 0.029	0.3902
Protein conversion efficiency	1.72 \pm 0.057	1.64 \pm 0.066	1.63 \pm 0.109	1.65 \pm 0.096	0.3902

★ Results are presented as mean \pm SD. Means in the same row with different superscript letters are significant ($p < 0$).

Muscle Composition of *H. Fossilis*

Table 3 presents the proximate muscle composition of Stinging catfish after a 60-day feeding trial with diets supplemented with varying concentrations of Si-NPs. The moisture content analysis showed significant differences between treatments, with T₀ having the highest moisture content (78.09 ± 0.005%) and T₁ the lowest (77.28 ± 0.005%). T₂ and T₃ exhibited intermediate moisture levels of 77.54 ± 0.005% and 77.86 ± 0.005%, respectively (p < 0.001). Regarding crude lipid content, T₂ recorded the highest value (3.33 ± 0.005%), significantly higher than T₀ (3.15 ± 0.005%) and the other groups. T₁ and T₃ had lower lipid levels of 2.88 ± 0.005% and 2.92 ± 0.025%, respectively (p < 0.001).

The crude protein content was significantly affected by Si-NP supplementation, with T₁ and T₂ showing the highest values (15.33 ± 0.025% and 15.32 ± 0.005%, respectively), while T₀ and T₃ exhibited lower protein levels at 14.88 ± 0.005% and 14.46 ±

0.005%, respectively (p < 0.001). For ash content, T₁ had the highest value (2.95 ± 0.03%), significantly greater than T₀ (2.23 ± 0.015%) and the other groups. T₀, T₂, and T₃ showed similar ash contents of 2.23 ± 0.015%, 2.21 ± 0.0075%, and 2.27 ± 0.008%, respectively (p < 0.001). Furthermore, the crude fiber content increased with higher Si-NP doses, with T₃ having the highest value (1.58 ± 0.005%) and T₀ the lowest (1.26 ± 0.01%). T₁ and T₂ exhibited intermediate values of 1.39 ± 0.005% and 1.46 ± 0.005%, respectively (p < 0.001). Similarly, carbohydrate content showed significant differences across treatments, with T₃ exhibiting the highest level (0.91 ± 0.01%), while T₀, T₁, and T₂ had carbohydrate contents of 0.41 ± 0.005%, 0.16 ± 0.005%, and 0.14 ± 0.005%, respectively (p < 0.001). These results demonstrate that dietary Si-NP supplementation significantly influences the muscle composition of *H. fossilis*, particularly affecting moisture, lipid, protein, ash, fiber, and carbohydrate contents in a dose-dependent manner.

Table 3: Final muscle composition of *H. fossilis* after 60 days of feeding trial

Treatments	T ₀	T ₁	T ₂	T ₃	Significance
Moisture	78.09 ± 0.005 ^a	77.28 ± 0.005 ^d	77.54 ± 0.005 ^c	77.86 ± 0.005 ^c	<0.001
Crude lipid	3.15 ± 0.005 ^b	2.88 ± 0.005 ^c	3.33 ± 0.005 ^a	2.92 ± 0.025 ^c	<0.001
Crude protein	14.88 ± 0.005 ^c	15.33 ± 0.025 ^a	15.32 ± 0.005 ^a	14.46 ± 0.005 ^c	<0.001
Ash	2.23 ± 0.015 ^b	2.95 ± 0.03 ^a	2.21 ± 0.0075 ^b	2.27 ± 0.008 ^b	<0.001
Crude fiber	1.26 ± 0.01 ^d	1.39 ± 0.005 ^c	1.46 ± 0.005 ^b	1.58 ± 0.005 ^a	<0.001
Carbohydrate	0.41 ± 0.005 ^b	0.16 ± 0.005 ^c	0.14 ± 0.005 ^c	0.91 ± 0.01 ^a	<0.001

★ Results are presented as mean ± SD. Means in the same row with different superscript letters are significant (p < 0.05)

4. DISCUSSION

Nanoparticles, as a part of nanotechnology, have become very popular in the aquaculture industry in the very recent years due to their positive role regarding the growth, immunity, disease resistance and overall well-being of several farmed aquaculture species (Khalefa et al., 2024; Mahboub et al., 2024). Several nanoparticles are used in different aquatic animals throughout the globe. The application of Si-NPs has significantly improved the growth of stinging catfish in the current study. Many studies have demonstrated that the provision of Si-NPs in the diet remarkably enhanced the growth performance of farmed fish species, including rohu, *Labeo rohita* (Murshed et al., 2023) and Nile tilapia, *Oreochromis niloticus* (Bashar et al., 2021). This growth increment could be attributed to the nutrient-carrying capacity of Si-NPs that may play a role in the better digestion and absorption of essential nutrients through controlled encapsulation and enhance their availability to the associated fish species (Bahabadi et al., 2017). In addition, NPs are crucial in improving gut microbial activity, which may contribute to better nutrient utilization (Onuegbu et al., 2018). However, a specific investigation is required with Si-NPs. Moreover, enhanced nutrient digestibility, especially protein digestibility, by dietary Si-NPs could also contribute to the growth increment in the current fish species (Bashar et al., 2021). Fish primarily obtain their metabolic energy from protein, specifically amino acids (Walton & Cowey, 1982; Wu et al., 2020). Lipids are stored in fish liver and muscles as a backup source if no adequate energy is found through protein (Kim et al., 2012; Zhang et al., 2019). Si-NPs undoubtedly increase digestibility and absorption of nutrients, including proteins and lipids (Bashar et al., 2021). However, higher levels of Si-NPs could negatively affect the growth performance of fish due to toxicity (Rashidian et al., 2023).

In the current study, dietary Si-NPs significantly enhanced the muscle protein concentration in Stinging catfish. Asad et al. (2023) and El-Shenawy et al. (2019) also observed protein increment in the muscle of *Labeo rohita* and *Oreochromis niloticus*, respectively, due to the incorporation of iron (Fe) nanoparticles in the diet. This may be a result of the enhanced protein adsorption ability of nanoparticles that can enhance protein linking, which results in enhanced protein retention in the fish muscle (Asad et al., 2023). Dietary Si-NPs likely also improved the muscle lipid level in the current study. Similar results have also been found in *Clarias batrachus* (Akter et al., 2018), and *Labeo rohita* (Asad et al., 2023) due to the provision of Fe-NPs in their diet. This could be attributed to the lipoprotein binding ability of nanoparticles in the blood, which resulted in higher lipid metabolism as well as enhanced lipid retention in the fish muscle (Asad et al., 2023).

CONCLUSION

In summary, dietary Si-NPs positively ameliorate the growth performance and fish muscle composition of Stinging catfish, and the best result was observed at 2 mg/kg Si-NPs. This level of Si-NPs can be effectively recommended as an important dietary supplementation for the better production of Stinging catfish as well as feed manufacturing companies associated in the preparation of fish feed. However, further investigations are recommended to determine the role of dietary Si-NPs on digestive enzyme activities, immune status and disease resistance of the host fish species.

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Author Contributions

P.S.D., was responsible for investigation, data analysis, and writing. M.F.R., Methodology, formal analysis, writing –editing, supervision, B.S.A.A.S., contributed in writing and resources, S.S.N., investigation and data curation. R.A.J., formal analysis. A.S., contributed investigation and resources. M.S.H., writing, and editing; and S.S.I. assisted with writing. All authors read and approved the final manuscript.

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