DIETARY CHITOSAN AND NANO-CHITOSAN OF BLACK SOLDIER FLY LARVAE FOR IMPROVING GROWTH AND PHYSIOLOGICAL INDICES OF
Clarias gariepinus

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ABSTRACT

This research examined the effect of nutritional supplementation of 5% chitosan and nano-chitosan sourced from black soldier fly larvae exuviae on the growth and physiological profile of *Clarias gariepinus* (initial weight: 2.054 ± 0.02 g) over a period of 30 days. The feed treatments consisted of chitosan-supplemented, nano-chitosan-supplemented, and control feed, arranged in triplicate. In each trial unit, 30 fish were reared in plastic tanks (60 L capacity, filled with 50 L of freshwater). Growth, hematological profiles, and antioxidant activities were assessed after 30 days. The findings indicated that nano-chitosan markedly improved growth performance, as shown by increased final weight, body weight gain (BWG), and feed efficiency (FE), in comparison to chitosan and control diets. Specifically, nano-chitosan-fed fish exhibited a final weight of 2.878 ± 0.16 g ind^{-1} and a feed conversion ratio (FCR) of 1.376 ± 0.15, outperforming the chitosan group (2.660 ± 0.12 g ind^{-1}; 1.267 ± 0.12) and the control group (2.344 ± 0.04 g ind^{-1}; 1.857 ± 0.05). Additionally, nano-chitosan significantly increased activities of antioxidant enzymes, including those of superoxide dismutase (SOD) and catalase (CAT), while reducing malondialdehyde (MDA) levels, indicating reduced oxidative stress. The hematological-profile remained stable across the groups, confirming the safety of these feed additives. This research emphasizes nano-chitosan as a viable sustainable food additive for improving growth rate and oxidative resistance in *C. gariepinus*.

KEYWORDS: BSF; chitosan; *Clarias gariepinus*; growth; nano-chitosan; survival

ABSTRAK: Pemberian Kitosan dan Nano-Kitosan dari Pupa Lalat Tentara Hitam melalui Pakan untuk Meningkatkan Pertumbuhan dan Kinerja Fisiologis Clarias gariepinus

*Penelitian ini mengevaluasi pengaruh suplementasi diet dengan 5% kitosan dan nano-kitosan dari cangkang pupa lalat tentara hitam terhadap pertumbuhan serta indeks fisiologis **Clarias***

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*gariepinus (berat awal: 2,054 ± 0,02 g) selama 30 hari. Perlakuan pakan terdiri atas pakan yang disuplementasi nano-kitosan, disuplementasi kitosan, dan kontrol yang diulang tiga kali. Pada masing-masing unit percobaan, 30 ekor ikan dipelihara di wadah plastik (kapasitas 60 L, diisi dengan air sebanyak 50 L). Setelah 30 hari pemeliharaan, pertumbuhan, profil hematologi, dan aktivitas antioksidan dievaluasi. Hasil penelitian menunjukkan bahwa nano-kitosan meningkatkan kinerja pertumbuhan, yang ditunjukkan dengan peningkatan berat akhir, pertambahan bobot tubuh (PBT), dan efisiensi pakan (EP), dibanding dengan pakan kitosan dan kontrol. Secara khusus, ikan yang diberi pakan nano-kitosan menunjukkan bobot akhir 2,878 ± 0,16 g ekor⁻¹ dan rasio konversi pakan (RKP) 1,376 ± 0,15, lebih tinggi dari kelompok kitosan (2,660 ± 0,12 g ekor⁻¹; 1,267 ± 0,12) dan kelompok kontrol (2,344 ± 0,04 g ekor⁻¹; 1,857 ± 0,05). Selain itu, nano-kitosan secara signifikan meningkatkan aktivitas enzim antioksidan, termasuk superoksida dismutase (SOD) dan katalase (CAT), sekaligus menurunkan kadar malondialdehida (MDA), yang mengindikasikan berkurangnya stres oksidatif. Parameter hematologi tetap stabil di seluruh kelompok, yang menegaskan keamanan bahan tambahan pakan ini. Penelitian ini menyoroti nano-kitosan sebagai suplemen pakan berkelanjutan yang menjanjikan untuk meningkatkan laju pertumbuhan dan ketahanan oksidatif pada **C. gariepinus.***

*KATA KUNCI: BSF; Clarias gariepinus; **kelangsungan hidup; kitosan; nano-kitosan; pertumbuhan***

INTRODUCTION

The African catfish (*Clarias gariepinus*) is a freshwater fish species with significant promise for aquaculture (Kebtieneh *et al.*, 2024; Tiamiyu *et al.*, 2023). This fish species has been commercially cultured under various farming settings and still demonstrates significant growth, despite being farmed in high-density conditions (Lisachov *et al.*, 2023; Thirukanthan *et al.*, 2023). Globally, catfish are the seventh most produced type of farmed finfish in terms of culture. Vietnam is currently the top global producer and exporter of farmed striped catfish, which is scientifically known as *Pangasianodon hypophthalmus*. Annually, Vietnam produces 1,400,000 tons of this fish from approximately 7,000 hectares of land. Similarly, China has cultivated the non-indigenous channel catfish (*Ictalurus punctatus*) as a significant agricultural product, yielding an annual production of 250,000 tons. In contrast, the main catfish species farmed in Africa, *C. gariepinus*, has a poor annual production of only 240,000 tons across the entire continent (Barasa & Ouma, 2024).

The increasing market demand for catfish has motivated aquaculture entrepreneurs to pursue optimal production of catfish to keep up with the demand (Isyagi, 2007; Pasch & Palm, 2021). However, catfish production is strongly influenced by a balanced relationship between the survival and growth of catfish and the quality of fish feed (Ayele, 2015; Stone *et al.*, 2024). Various initiatives have been undertaken to improve the quality of fish diets, which are cost-effective, readily available, and sustainable, thereby enhancing the survival and growth indices of fish. An approach to enhance the quality of fish diets is to add chitosan from the exuviae of black soldier fly (*Hermetia illucens* L.) larvae.

The black soldier fly (BSF) is an insect that is very easy to find and abundant (Nugroho *et al.*, 2023). The BSF larvae (BSFL) are often utilized as organic waste-decomposing organisms to reduce the accumulation of organic waste (Siddiqui *et al.*, 2022). Besides being a decomposing organism, BSFL is also used as feed for farm animals, particularly fish and chicken (de Souza Vilela *et al.*, 2023; Maroušek *et al.*, 2023). This is due to BSFL contains a

variety of nutrients, including fatty acids, minerals, and proteins (Lu *et al.*, 2022; Nugroho *et al.*, 2022; Seyedalmoosavi *et al.*, 2023). The protein content (dry base) produced by the BSFL is 41.44 g per 100 g, and the fat content of 35.69 g per 100 g (Ebeneezar *et al.*, 2021).

Although rich in protein and lipid contents (Nugroho *et al.*, 2024a), the pupae shell of BSFL (exuviae) also contains chitosan (Guarnieri *et al.*, 2022; Khayrova *et al.*, 2019; Triunfo *et al.*, 2022). Chitosan has the potential to be used as a supplementary feed ingredient in fish diets (Abdel-Ghany & Salem, 2020), which can improve growth performance (Chen & Chen, 2019), antioxidant activity (Yu *et al.*, 2023), and hematological profile of farmed fish (Salam *et al.*, 2021). To date, research on chitosan derived from crab shells, fish scales, and shrimp shells has been conducted to enhance the antioxidant activity of feeds (Kumari *et al.*, 2017; Sarbon *et al.*, 2015).

Currently, research related to chitosan and its benefits is very popular and has been applied in the health (Aryani *et al.*, 2023) and aquaculture sectors (Nugroho *et al.*, 2024b). Furthermore, the application of nanotechnology in chitosan research (nanogel) has been conducted on tilapia feed. One of the main findings of the current research subject of investigation indicated that chitosan nanogel improved the blood profile, blood biochemistry, and antioxidant response in tilapia fish (Mahboub *et al.*, 2024). The impacts of chitosan-based nanoparticles on the growth indices, antioxidant defenses, and immune responses of tilapia (*Oreochromis niloticus*) have been evaluated, demonstrating beneficial outcomes for the aquafeed industry (Abdel-Tawwab *et al.*, 2019).

Several studies on chitosan and nano-chitosan supplementation (from crab, shrimp, and fish shells) in fish pellets and their effects on blood profile, growth, and antioxidant activity in various fish species have been explored (Abdel-Ghany & Salem, 2020; El-Naggar *et al.*, 2021; Ismael *et al.*, 2021). However, the supplementation of chitosan or nano chitosan from BSFL exuviae on the growth, blood profile, and antioxidant activity

of *C. gariepinus* has not been performed. Thus, the current research aimed to assess the effect of nutritional supplementation to the diet, either chitosan or nano-chitosan, on the growth indices, hematological profile, and antioxidant activities of African catfish.

MATERIALS AND METHODS

Chitin Extraction

The chitosan extraction procedure was conducted following an earlier method published by Lagat *et al.* (2021) with minor adjustments. The pupal shells of BSF, or exuviae, were sorted and rinsed under running water until clean, then dried at 60°C for 24 hours using a food dehydrator. One hundred g of dried-purified-exuviae (100 g) were weighed using an analytical balance (A&D, model ER-180A, Tokyo, Japan) and then immersed in 1,000 mL of 2% sodium hydroxide (NaOH) (Pudak Scientific, Bandung, Indonesia) for 2 hours with intermittent stirring to eliminate protein. Subsequently, the exuviae were meticulously rinsed with distilled water to achieve a neutral pH. The pH value was determined using an Orion Star™ A211 Benchtop pH meter (Waltham, MA, USA). The exuviae were subsequently desiccated at 60°C for 24 hours using a food dehydrator (Model: LT-28, China). The calcium carbonate was removed from the desiccated samples by immersing them in 1,000 mL of 7% HCl for 4 hours at ambient temperature with intermittent agitation. The completed product was thoroughly rinsed with distilled water until reaching a neutral pH and subsequently dried using a food dehydrator at 60°C for 24 hours. The desiccated finished product was weighed, and the percentage yield of chitin was computed based on the exuviae utilized.

Chitosan Production (Deacetylation)

Chitin obtained from exuviae (100 g) was subsequently transformed into chitosan by immersion in 1,000 mL of 50% NaOH solution (Pudak Scientific, Bandung, Indonesia) at 120°C for 2 hours using the reflux system

(Hermiyati & Juhana, 2019; Junaidi *et al.*, 2009). The deacetylated chitosan was subsequently purified with distilled water until a neutral pH was achieved and then dehydrated in a food dehydrator at 60°C for 24 hours. The desiccated samples were preserved at 4°C in sealed plastic bags until required.

Yield Percentage

The dry weights of exuviae, chitin, and chitosan obtained after extraction were utilized to calculate the yields of chitin and chitosan, according to the following formula (1) and (2) (Lagat *et al.*, 2021):

$$\text{Chitin Yield (\%)} \quad = \quad \frac{a}{b} \text{ x100\%} \quad ....(1)$$

Where:
a represents the weight of the chitin (g)
b denotes the initial weight of exuviae (g)

$$\text{Chitosan Yield (\%)} \quad = \quad \frac{c}{d} \text{ x100\%} \quad ....(2)$$

Where:
c represents the weight of chitosan obtained (g)
d denotes the initial weight of exuviae (g)

The extent of deacetylation was assessed using the following formula (3) and (4), delineated by Sánchez-Machado *et al.* (2024):

$$\%DD = 100 - \%DA \quad ....(3)$$

$$\%DA \quad = \quad \left(\frac{A1655}{A3450} \right) \text{ x } \left(\frac{100}{1.33} \right) \quad ....(4)$$

%DD represents the percentage of deacetylation degree, %DA denotes the percentage of acetylation degree, while A1655 and A3450 signify the absorbance values at the infrared wavelengths of 1655 nm and 3450 nm, respectively.

Biosynthesis of Nano-chitosan

Chitosan nanoparticles were synthesized following the methods of Chávez de Paz *et al.* (2011) and Younus *et al.* (2020). In order to produce the chitosan solution, 0.25 g of chitosan was immersed in 1 mL of acetic acid. A 1.0 M NaOH solution was added to adjust the acidity of the solution to 5.5, and distilled water was then added to bring the volume to 100 mL. The mixture was agitated overnight. A bluish suspension (nanoparticles) was subsequently generated by adding sodium tripolyphosphate (STPP) dropwise at ambient temperature and maintaining continuous agitation. The mixture was subsequently centrifuged at 10,000 rpm for 10 minutes, after which the supernatant was carefully decanted, leaving the nanoparticles at the bottom. The nano-chitosan that was produced was subsequently freeze-dried.

Test Diet Preparation

Control diet ingredients were purchased from a commercial feed in Samarinda, East Kalimantan, Indonesia. Table 1 displays the composition of the control diet. The control diet composition included protein levels between 38% and 42%, fat ranging from 4% to 6%, 2% raw fiber, 10% inorganic matter, and 12% water proportion. The test food was prepared by combining the control diet with 5% chitosan or nano-chitosan, pelletizing it (0.5 mm) using a mincer, and then drying it in an oven at 60°C for 24 hours. After drying, the pellets were allowed to cool to ambient temperature before being packaged and kept in a dark area.

Fish Rearing

African catfish (initial weight: 2.054 ± 0.02 g) were obtained from a fish farmer breeding at Samarinda, East Kalimantan, and acclimation was carried out for a week under laboratory conditions. During acclimatization, test fish were fed a basal feed. A total of 600 fish were randomly distributed among the trial units of control, chitosan, and nano-chitosan dietary treatment groups, with 30 fish allocated to each of 12 tanks (plastic containers). Each tank, with a volume of 60 L (filled with 50 L of freshwater), was connected to a recirculating aquaculture system. Water circulation was

Table 1. The ingredients of formulated diet supplemented with chitosan and nano-chitosan for *Clarias gariepinus*

Composition	g kg^{-1}
Fish meal	423.5
Soybean meal	282.0
Maize germ	109.80
Wheat bran	164.70
Fish oil	9.8
Vitamin premix	4.9
Mineral premix	4.9
Total	**1,000**

maintained using a Grundfos NSBasic 4-23 motor pump, ensuring a continuous flow rate of 0.54 L per min of oxygenated water. Fish in each treatment were fed diets at a rate of 3% of their body weight, administered three times daily over 30 days.

To maintain water quality, 30–50% of the tank water was renewed with freshwater three times per week. Key water quality parameters, including temperature, pH, total ammonia nitrogen, nitrite, and dissolved oxygen, were monitored weekly using a YSI 550A dissolved oxygen meter (Clandon, Ohio, USA), a Sera nitrite test kit (SERA GMBH, Germany), and a Eutech Instruments Cyberscan pH 11 meter.

Growth and Feeding Parameters

On day 30, measurements of average weekly gain (AWG), body weight gain (BWG), daily weight gain (DWG), feed conversion ratio (FCR), percentage of feed efficiency (FE), and survival rate (SR) were conducted to evaluate the growth performance and feed intake efficiency of treated fish. All growth-related metrics were calculated based on the formulas (5) to (9) (Abdel-Tawwab *et al.*, 2015; Githukia *et al.*, 2015):

BWG (g) = final weight of fish (g) – initial weight of fish (g) .... (5)

$$\text{AWG (g week}^{-1}) = \frac{\text{Body weight gain (g)}}{\text{Duration in weeks of study}} \quad ....(6)$$

$$\text{DWG (g day-1)} = \frac{\text{Body weight gain of fish (g)}}{\text{Number of experimental days}} \quad ....(7)$$

$$\text{FE (\%)} = \frac{[(\text{final biomass (g)} + \text{cumulative weight of dead fish (g)}) - \text{initial biomass (g)}]}{\text{Total feed intake (g, dry matter basis)}} \times 100 \quad .....(8)$$

$$\text{FCR} = \frac{\text{Total feed intake (g, dry weight)}}{\text{Total body weight gain (g)}} \quad ....(9)$$

At the final day of the study, the survival rate (SR) of fish in each tank was evaluated following the equation (10) (Okomoda *et al.*, 2017):

$$\text{SR (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\% \quad ....(10)$$

Fish Blood Collection

Blood sampling was performed by withdrawing blood from the dorsal aorta with a syringe (1 mL; 28G needle) positioned between the anus and anal fin. The obtained blood was placed in a microtube containing 0.2% EDTA (0.5 mL).

Hematological Analysis

On the 30th day of the experiment, blood already collected during the fish blood sampling was used for hematological analysis. The measurements of total red blood cells (RBC; 10^6 μL^{-1}), white blood cells (WBC;

10^3 μL^{-1}), hemoglobin concentration (Hb; g dL^{-1}), hematocrit (Htc), and platelet counts were conducted using an Auto Hematology Analyzer (Mindray BC2800, Mindray®, Shenzhen, China).

Antioxidant Activity

The antioxidant activity was measured using blood samples from the fish collected on the final day of the study. Superoxide dismutase (SOD) levels were determined using an SOD reagent kit (Solar Bio SOD Assay Kit Catalogue Number BC0170), while malondialdehyde (MDA) levels were measured using a Solar Bio MDA Assay Kit (catalog number BC0020).

Statistical Analysis

Data obtained from the study (including growth performance, blood profiles, and antioxidant activity) were analyzed using SPSS Statistics (version 24), and the results are shown as average ± standard errors. Statistically significant differences were identified by using Tukey's test with significance defined at $p < 0.05$ to assess differences among the control, chitosan-treated, and nano-chitosan-treated fish groups.

RESULTS AND DISCUSSION

Chitosan and Nano-chitosan Determination

Compared with traditional sources, BSFL exuviae have several advantages. Unlike crustacean shells, the BSFL exuviae have a lower inorganic content, which simplifies the demineralization process. This aligns with the findings of Aranaz *et al.* (2009), who reported faster and more efficient extraction processes for insect-derived chitin sources. Additionally, BSFL-based processes contribute to waste valorization and sustainability, echoing environmental benefits. Despite these advantages, the chitosan yield from BSFL (56.33%) was slightly lower than the maximum yield reported for shrimp shells (up to 65%) (Kumari *et al.*, 2017).

The yield data for chitin and chitosan from the BSFL exuviae revealed that the extraction process, which includes deproteinization, demineralization, and deacetylation, yields significant amounts of biopolymers (Table 2). The chitin yield obtained was 15.30 ± 6.86%, with variations attributed to the initial weight of BSFL exuviae and the efficiency of deproteinization and demineralization. Similarly, the chitosan yield observed was 13.57 ± 9.03%, highlighting the critical role of the deacetylation process in determining the final yield. This pattern demonstrates that the process efficiency improved with increased raw material input, suggesting the potential scalability of the method.

The present findings suggest that BSFL exuviae represent a viable alternative for chitin and chitosan production, yielding amounts similar to those derived from conventional sources, such as shrimp and crab shells. The observed chitin yields (15.30 ± 6.86%) were lower than the 20-40% range reported in previous studies, whereas the chitosan yields (13.57 ± 9.03%) fell below the 40-70% range (Elieh-Ali-Komi & Hamblin, 2016; Kumari *et al.*, 2017). The scalability of the process, as demonstrated by its higher efficiency with larger sample sizes, suggests its potential for industrial applications.

Furthermore, the FTIR results validate the chemical processes involved in extracting chitin and converting it into chitosan. The characteristic spectral features observed in this study were consistent with those reported in the literature. Barwin Vino *et al.* (2011) and Elieh-Ali-Komi and Hamblin (2016) highlighted similar changes in FTIR spectra, indicating successful deacetylation. The structural integrity and functional group transformations ensure that the produced chitosan is suitable for applications in biomedicine and industry.

The FTIR spectra of the fish feed diets (control diet, control diet with chitosan, and control diet with nano-chitosan) revealed distinct variations in the functional groups associated with the presence of chitosan

Table 2. Yield of chitin and chitosan derived from the black soldier fly larvae (BSFL) exuviae

Replication	Weight of BSFL exuviae (g)	Deproteination (%)	Demineralization (%)	Deacetylation (%)	Chitin yield (%)	Chitosan yield (%)
1	100	70.39	47.55	23.14	23.14	23.14
2	200	43.77	34.14	20.78	10.39	5.19
3	100	41.70	36.41	12.37	12.37	12.37
Average	133.33	51.95	39.37	18.76	15.30	13.57
Standard deviation	57.74	16.00	7.18	5.66	6.86	9.03
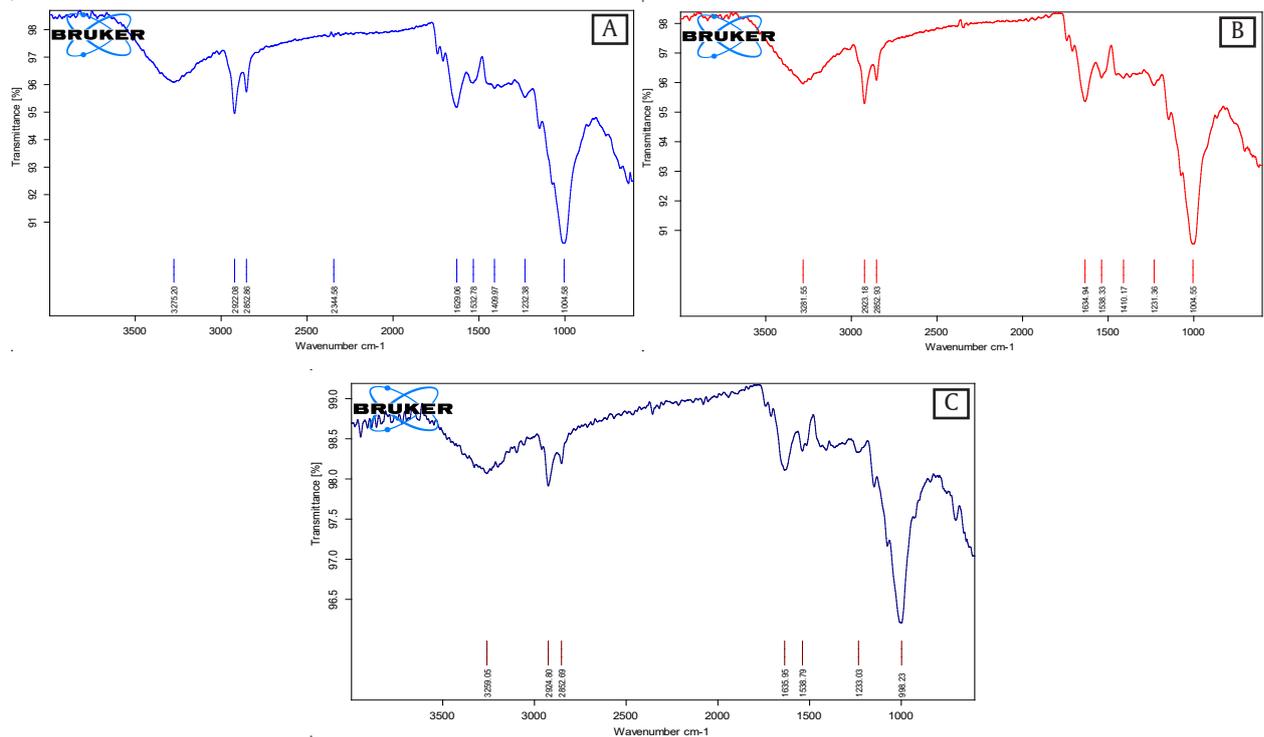
Figure 1. The fourier transmission infrared for fish feed containing chitosan derived from the black soldier fly larvae exuviae. A. Control feed; B. Control feed with chitosan; C. Control feed with nano-chitosan

and nano-chitosan (Figure 1). Control diet (A): The spectrum of the control diet exhibits characteristic peaks corresponding to its primary components. Peaks at 2922 cm^{-1} and 2852 cm^{-1} correspond to C-H stretching vibrations, while the amide I band appears at 1629 cm^{-1}, which is associated with proteins. No peaks indicative of chitosan or related functional groups were observed. Control diet with chitosan (B): The FTIR spectrum of diet B showed additional peaks attributed to chitosan. The amide I group (C=O stretching)

is observed around 1635 cm^{-1}, while a peak at approximately 1410 cm^{-1} indicates N-H bending and C-H deformation. A broad peak around 3281 cm^{-1} is due to O-H stretching, further confirming the presence of chitosan in the diet. Furthermore, control diet with nano-chitosan (C): The spectrum for diet C showed similar but more pronounced peaks. The amide I band shifted slightly to 1636 cm^{-1}, likely due to the reduced particle size of nano-chitosan. Additional peaks at 998 cm^{-1} and 1233 cm^{-1} highlight enhanced polysaccharide

characteristics, indicative of the structural and functional differences introduced by nano-chitosan.

Water Quality Parameters

Throughout the experimental period, water quality remained within optimal ranges suitable for the rearing of *C. gariepinus* (Table 3). All water quality parameters, such as temperature, pH, dissolved oxygen (DO), NO_2 (nitrate), and NH_3 (ammonia), supported the optimum growth and metabolism of *C. gariepinus*.

Growth Performance

The growth indices of *C. gariepinus* fed diets supplemented with 5% chitosan or nano-chitosan derived from BSFL exuviae for 30 days were significantly improved compared to the control diet. Key growth parameters of treatment groups (chitosan and nano-chitosan), including body weight gain (BWG), average weekly gain (AWG), daily weight gain (DWG), feed efficiency (FE), and feed conversion ratio (FCR), were significantly different from control (Table 4).

The nano-chitosan group exhibited the highest final weight (2.878 ± 0.16 g ind^{-1}), BWG (0.823 ± 0.33 g), AWG (0.027 ± 0.010 g week^{-1}), and DWG (0.027 ± 0.010 g day^{-1}), followed by the chitosan group with values of final weight (2.660 ± 0.12 g ind^{-1}), BWG (0.450 ± 0.15 g), AWG (0.015 ± 0.005 g week^{-1}), and DWG (0.015 ± 0.005 g day^{-1}), respectively. The control group demonstrated the lowest values for these parameters, with a final weight of 2.344 ± 0.04 g^{-1}, BWG of 0.263 ± 0.02 g, AWG of 0.008 ± 0.001 g week^{-1}, and DWG of 0.008 ± 0.001 g day^{-1}. Feed efficiency (FE) improved significantly in both chitosan (80.26 ± 7.23%) and nano-chitosan (74.63 ± 5.15%) groups compared to the control (53.91 ± 1.65%), though no significant difference was observed between the chitosan and nano-chitosan groups for this parameter. Similarly, the feed conversion ratio (FCR) was significantly better in the chitosan (1.267 ± 0.12) and nano-chitosan (1.376 ± 0.15) groups than in the control group (1.857 ± 0.05). Survival rates (SR) were high and did not differ significantly among groups, remaining above 93% across all treatments.

In general, the results demonstrated that the inclusion of BSFL-derived nano-chitosan in the diet significantly enhanced the growth performance and feed utilization of catfish compared with chitosan and the control diet. The observed trends were aligned with the biofunctional properties of nano-chitosan, such as enhanced nutrient absorption and immunomodulatory effects. The superior performance of the nano-chitosan group in terms of final weight, BWG, AWG, and DWG can be attributed to the nanoscale particle size, which increases nutrient bioavailability. These findings are similar to those of Jeyakumari *et al.* (2016), who reported improved growth rates in *Pangasius hypophthalmus* fed chitosan-enriched diets. Similarly, a previous study found that nano-chitosan supplementation in tilapia diets enhanced growth performance by promoting intestinal nutrient absorption and reducing oxidative stress (El-Naggar *et al.*, 2021; Hossam-Elden *et al.*, 2024).

The enhanced feed efficiency and lower FCR observed in the nano-chitosan group were consistent with the findings of previous studies. For instance, Abdel-Tawwab *et al.* (2019) and Abd El-Naby *et al.* (2019) demonstrated that nano-chitosan improves feed conversion in *O. niloticus*, likely because of its antimicrobial properties and its ability to stimulate beneficial gut microbiota. In contrast, fish fed chitosan in their diet showed moderate effects on feed efficiency, reflecting its lower bioavailability compared to its nano counterpart.

Furthermore, the survival rates across all groups were similar, suggesting that both chitosan and nano-chitosan can be safely included in catfish diets. A previous study reported that dietary chitosan supplementation did not negatively affect survival rates in fish, further supporting the safety of these

Table 3. Water quality parameters during the study on *Clarias gariepinus* fed chitosan or nano-chitosan in the diet for 30 days

Treatments	Temperature (°C)	pH	Dissolved oxygen (mg L^{-1})	NO$_2$	NH$_3$
Control	27.8	7.31	7.65	0.4	0
Chitosan	27.8	7.31	7.65	0.4	0
Nano-chitosan	27.8	7.31	7.65	0.4	0

Table 4. Growth parameters of *Clarias gariepinus* fed chitosan or nano-chitosan in the diet for 30 days

Parameters	Control	Chitosan	Nano-chitosan
Initial weight (g ind-1)	2.085 ± 0.02[a]	2.212 ± 0.04[a]	2.057 ± 0.09[a]
Final weight (g ind-1)	2.344 ± 0.04[a]	2.660 ± 0.12[b]	2.878 ± 0.16[c]
BWG (g)	0.263 ± 0.02[a]	0.450 ± 0.15[b]	0.823 ± 0.33[c]
AWG (g week-1)	0.008 ± 0.001[a]	0.015 ± 0.005[b]	0.027 ± 0.010[c]
DWG (g day-1)	0.008 ± 0.001[a]	0.015 ± 0.005[b]	0.027 ± 0.010[c]
FE (%)	53.91 ± 1.65[a]	80.26 ± 7.23[b]	74.63 ± 5.15[b]
FCR	1.857 ± 0.05[b]	1.267 ± 0.12[a]	1.376 ± 0.15[a]
SR (%)	97.84 ± 1.07[a]	94.62 ± 2.84[a]	93.54 ± 4.92[a]

Note: Results are shown as average ± standard error (SE). Distinct superscripts within the same row indicate significant differences (*p < 0.05*). The following abbreviations are used: BWG represents body weight gain, AWG stands for average weekly gain, DWG refers to daily weight gain, FE denotes feed efficiency, FCR is the feed conversion ratio, and SR indicates survival rate.

additives (Wang & Li, 2011). While the current study highlights the benefits of nano-chitosan, previous research has revealed species-specific and dose-dependent variations in its effects. For example, Younus *et al.* (2020) found that nano-chitosan improved the growth and feed efficiency of *Labeo rohita*, but excessive doses had minimal benefits.

Hematological Profile

The hematological parameters examined in this study provide insights into the physiological and immunomodulatory effects of dietary BSFL-derived chitosan on catfish. Despite the lack of statistically significant differences, the observed trends suggest that chitosan supplementation, particularly in the nanoform, may influence fish health. The hematological profile of catfish fed diets containing micro- or nano-chitosan from BSFL exuviae was analyzed after 60 days. The results, summarized in Table 5, include parameters such as red blood cell (RBC) count, hemoglobin concentration (Hb), white blood cell (WBC) count, and platelet (PLT) count.

The control group exhibited the highest RBC count (0.406 ± 0.031 × 10⁣ μL^{-1}) compared with the chitosan and nano-chitosan groups. The Hb levels in the chitosan and nano-chitosan groups were numerically lower than control, with the nano-chitosan group showing slightly reduced Hb (2.666 ± 0.424 g dL^{-1}) values compared to the control group. However, these differences were not statistically significant (*p > 0.05*). The WBC count was highest in the fish in the chitosan group (0.510 ± 0.151 × 10³ μL^{-1}), followed by the control group (0.336 ± 0.263 × 10³ μL^{-1}), and the nano-chitosan group (0.190 ± 0.140

Table 5. Hematological profile of *Clarias gariepinus* fed chitosan or nano-chitosan in the diet for 30 days

Parameters	Control	Chitosan	Nano-chitosan
RBC (10^6 uL^{-1})	0.406 ± 0.031[b]	0.233 ± 0.030[ab]	0.193 ± 0.015[a]
Hb (g dL^{-1})	2.796 ± 0.859[a]	2.2433 ± 0.596[a]	2.666 ± 0.424[a]
WBC (10^3 uL^{-1})	0.336 ± 0.263[b]	0.510 ± 0.151[c]	0.190 ± 0.140[a]
PLT (10^3 uL^{-1})	204.90 ± 150.400[c]	116.900 ± 11.960[a]	197.633 ± 63.480[b]

Note: The data are expressed as average ± standard error (SE). Superscripts within the same row signify significant differences ($p < 0.05$). The abbreviations used are as follows: RBC for red blood cell, Hb for hemoglobin, WBC for white blood cell, and PLT for platelet.

$\times 10^3 \mu L^{-1}$). The platelet count was highest in the control group (204.90 ± 150.400 × 10^3 μL^{-1}), followed by the nano-chitosan group (197.633 ± 63.480 × 10^3 μL^{-1}). Fish in the chitosan group exhibited the lowest platelet count (116.900 ± 11.960 × 10^3 μL^{-1}).

The reduction in RBC observed in the nano-chitosan group was consistent with past findings that dietary nano-chitosan did not significantly enhance RBC in *O. niloticus*, but remained stable under normal physiological conditions (Chellapandian *et al.*, 2023; El-Naggar *et al.*, 2021). Furthermore, the highest WBC count in the chitosan group was also consistent with a study performed by Niu *et al.* (2013), who found that chitosan could stimulate immune cell production, particularly under moderate oxidative stress conditions. However, the nano-chitosan group's lower WBC count in this study contrasts with studies reporting enhanced immune responses with nano-chitosan (Oushani *et al.*, 2020). This discrepancy may be due to differences in nano-chitosan particle size or species-specific immune response. The platelet counts in this study were differed between control and treatments groups, in contrast to the findings of Busilacchi *et al.* (2013), who reported that dietary chitosan does not significantly influence platelet production, but helps maintain hemostasis. The reduction in the chitosan group compared with the control and nano-chitosan groups suggests possible differences

in bioavailability or uptake efficiency.

The observed hematological stability suggests that BSFL-derived chitosan supplementation, particularly in nano-form, may serve as a safe and sustainable feed additive for aquaculture species. Nano-chitosan's nanoscale properties likely enhance cellular uptake and metabolic interactions, potentially influencing immune modulation and stress responses. However, the limited numerical differences across groups suggest that further research is needed to optimize dosage and assess the long-term impacts.

Antioxidant Activity

The present study evaluated the antioxidant activity of catfish following dietary supplementation with chitosan and nano-chitosan derived from BSFL exuviae. The superior performance of nano-chitosan may stem from its enhanced absorption and interaction with cellular antioxidant systems such as superoxide dismutase (SOD) and catalase. This hypothesis is supported by earlier reports suggesting that nanoformulations improve intracellular uptake and antioxidant gene expression. The results showed notable differences between the treatment groups. The antioxidant activity, measured through specific biochemical assays, revealed that dietary inclusion of nano-chitosan led to a statistically significant increase ($p < 0.05$) in antioxidant

activity compared to both chitosan and the control group.

The results showed an increase in SOD activity in fish fed diets supplemented with nano-chitosan compared with the control diet. However, The SOD activity of fish in chitosan group was similar to the nano-chitosan and control group (Figure 2). The catalase (CAT) activity followed a similar trend as SOD, with nano-chitosan supplementation eliciting the highest response. The CAT of fish in the chitosan group showed no significant difference ($p > 0.05$) compared to the control, but these were less pronounced than those in the nano-chitosan group (Figure 3). The MDA, a marker of lipid peroxidation, was significantly reduced ($p < 0.05$) in both chitosan-supplemented groups, with the nano-chitosan group exhibiting a lower level than control group. The reduction in MDA suggests that dietary supplementation mitigates oxidative stress, with nano-chitosan providing superior protection (Figure 4).
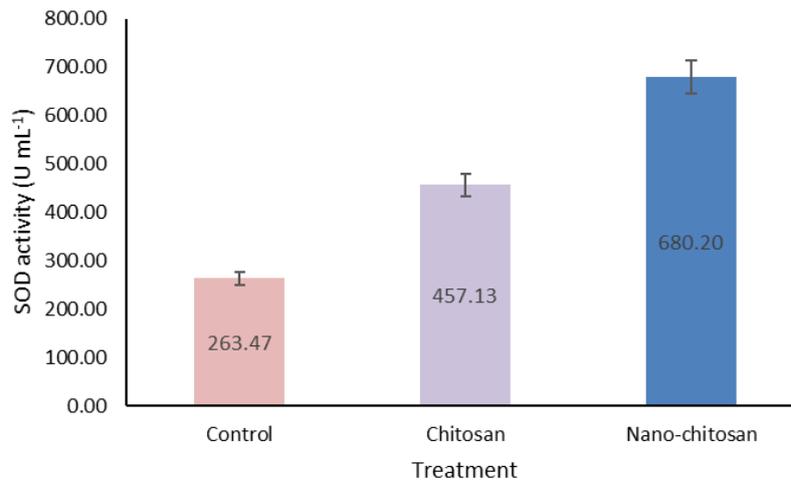

Figure 2. Superoxide dismutase (SOD) activity of the *Clarias gariepinus* fed either chitosan or nano-chitosan supplemented diet for 30 days. The same letter above the error bar indicated no significant difference at the level $p > 0.05$
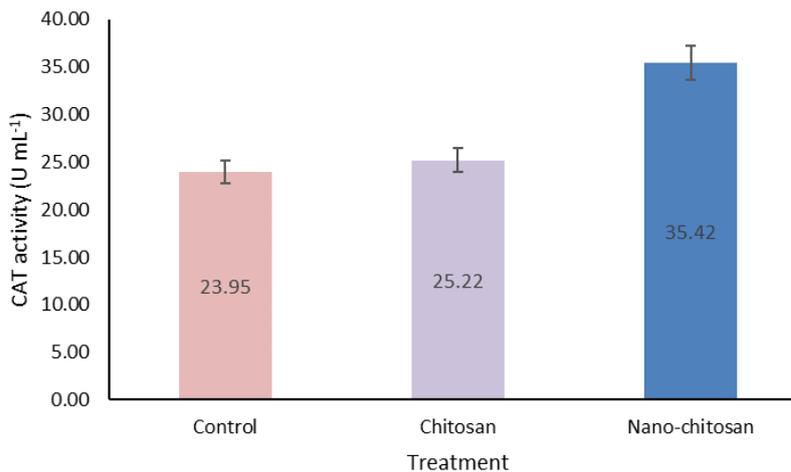

Figure 3. Catalase activity of the *Clarias gariepinus* fed either chitosan or nano-chitosan supplemented diet for 30 days. The same letter above the error bar indicated no significant difference at the level $p > 0.05$
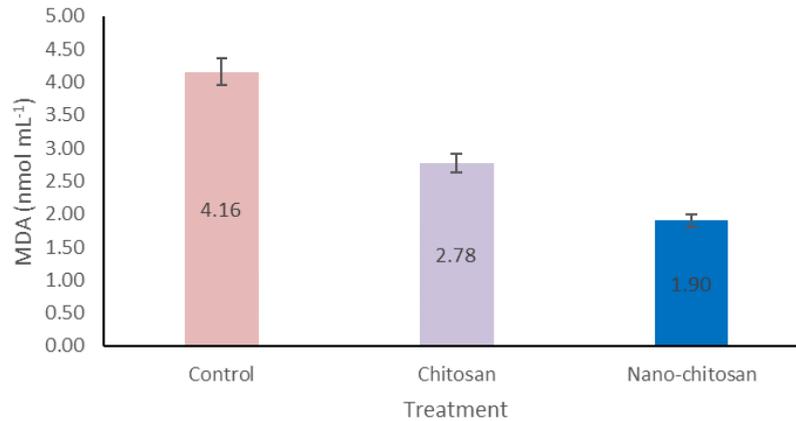
Figure 4. Malondialdehyde activity of the *Clarias gariepinus* fed either chitosan or nano-chitosan supplemented diet for 30 days. The same letters above the error bar indicated no significant difference at the level $p > 0.05$

The enhancement in SOD activity observed in the present study is consistent with previous research, which demonstrates that nano-chitosan improves antioxidant enzyme activities in *O. niloticus* (Abd El-Naby *et al.*, 2019; Abdel-Tawwab *et al.*, 2019; Hossam-Elden *et al.*, 2024). This improvement was attributed to the higher bioavailability and interaction of nano-chitosan with cellular enzymes. Meanwhile, the enhanced CAT activity observed in this study was comparable to the findings of Hussein *et al.* (2021), who reported an increased CAT level in shrimp fed nano-chitosan-enriched diets. This similarity highlights the broader applicability of nano-chitosan across various species. However, the magnitude of improvement in catfish suggested species-specific differences in metabolic responses to chitosan supplementation. In contrast, the significant reduction in MDA levels confirmed the antioxidant role of nano-chitosan, indicating decreased lipid peroxidation in fish under oxidative stress when fed diets containing bio-based nanomaterials (Ibrahim *et al.*, 2021).

The nano-chitosan's higher efficacy is likely due to its nanoscale properties, such as increased surface area and reactivity, which facilitate better interaction with oxidative pathways. Nano-chitosan may enhance the expression or activity of antioxidant enzymes, such as SOD and CAT, while scavenging reactive oxygen species (ROS) and reducing oxidative damage, as evidenced by decreased MDA levels. The current study underscores the potential of nano-chitosan as a dietary supplement to enhance antioxidant defenses in aquaculture. These findings support sustainable aquaculture practices, particularly leveraging insect-derived bioactive compounds.

CONCLUSION

The conclusion of this study shows that *C. gariepinus* fed nano-chitosan grew significantly better than those on standard or chitosan diets, with an improved final weight, body weight gain, average weekly gain, daily weigh gain, and antioxidant responses. This study highlights the feasibility of using insect-derived nano-chitosan to boost aquaculture productivity, offering a cost-effective and environmentally friendly solution.

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AUTHOR CONTRIBUTION

NM contributed to the experimental activities, literature analysis, data collection, and writing of the manuscript. RA performed literature analysis, experimental activities, writing of the manuscript, and critically analyzed the manuscript. RR collected, handled, and validated the data. HM prepared experimental activities, data handling, and validation. RAN performed the experiments and wrote the manuscript.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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