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Harnessing Nano-Chitosan for Sustainable Aquaculture: Enhanced Growth, Feed Utilization, and Profitability in the Nile Tilapia (*Oreochromis niloticus* L.)

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ABSTRACT

This study evaluates the impact of dietary Nano-chitosan supplementation on the growth performance, feed utilization, body composition, energy retention, protein productive value (PPV%), and economic efficiency of the Nile tilapia (*Oreochromis niloticus*) fingerlings. Thus, a total of 168 fish were acclimated and randomly distributed into 12 aquaria (14 fish per aquarium) with an initial average weight of 102 ± 0.48 g. Four iso-nitrogenous and iso-caloric diets were formulated to contain Nano-chitosan at 0g/ kg (G1), 2g/ kg (G2), 4g/ kg (G3), and 6g/ kg (G4). Fish fed the diet containing 2g Nano-chitosan/kg (G2) showed significant improvements in final body weight, total body weight gain, average daily gain, specific growth rate, relative growth rate, feed intake, crude protein intake, and protein efficiency ratio, along with a markedly improved feed conversion ratio. Nano-chitosan supplementation also influenced the fish's body composition: G3 recorded the highest crude protein content, followed by G4 and G2, whereas G1 exhibited the highest ether extract value. Similarly, G3 achieved the highest gross energy content, followed by G4. Both energy retention (%) and PPV% were notably enhanced in G2. Economic evaluation indicated that the 2g/ kg Nano-chitosan diet achieved the most favorable feed cost efficiency and profitability. Overall, the findings demonstrate that incorporating Nano-chitosan at low dietary levels particularly 2g/ kg can significantly improve growth performance, feed utilization, nutrient retention, and economic return in the Nile tilapia aquaculture, highlighting its potential as a sustainable feed additive.

INTRODUCTION

Aquaculture plays a vital role in meeting the growing global demand for fish and other aquatic organisms, serving as a primary source of high-quality animal protein for human nutrition (**FAO, 2020**). Over recent decades, the aquaculture sector has experienced rapid expansion, driven by the need to enhance fish nutrition and develop more sustainable production systems (**FAO, 2018**). This ongoing progress is essential to ensure global nutritional security, as highlighted by **Fajardo *et al.* (2022)**, who emphasized the importance of continuous innovation to improve productivity, efficiency, and sustainability in aquaculture practices.

Nanotechnology has emerged as a promising tool in this context, offering solutions that enhance production efficiency and minimize environmental impacts. **Rathore and Mahesh (2021**) defined nanotechnology as the manipulation of materials at a nanoscale (1– 100nm), which significantly alters their physicochemical properties due to increased surface area and reactivity. Its applications in the fisheries sector are extensive, ranging from water treatment and sterilization to disease control and the development of Nano-feeds (**Fath El-Bab *et al*., 2020**).

Among the various aquaculture species, *Oreochromis niloticus* stands out as one of the most widely cultured freshwater fish worldwide, known for its rapid growth, efficient feed conversion, high market value, and consumer acceptance (**El-Sayed, 2019**). The continuous development of effective feed additives remains crucial to improve fish performance, health, and sustainability in tilapia farming systems.

Chitosan, a natural polysaccharide derived from the deacetylation of chitin found in crustacean shells, insects, and fungi, has gained considerable attention as an effective immunostimulant in aquaculture (**Kou *et al*., 2021**). Its advantageous characteristics—such as low toxicity, biodegradability, biocompatibility, and cost-effectiveness—make it particularly suitable for feed applications (**Muzzarelli, 2010; Mohammed *et al*., 2017**). Moreover, chitosan exhibits antibacterial, antioxidant, and immune-enhancing properties, contributing to improved fish growth and disease resistance while reducing water pollution (**Abu-Elala *et al*., 2015; Aranaz *et al*., 2021**).

Recent advancements have led to the development of chitosan nanoparticles (CsNPs), which possess superior functional properties compared to conventional chitosan due to their small particle size, large surface area, and high bioavailability (**Aranaz *et al*., 2009; Augustine *et al*., 2019**). These nanoparticles protect active compounds from degradation in the gastrointestinal tract, enhance nutrient absorption, and can serve as efficient carriers for bioactive substances in feed formulations (**Alishahi *et al*., 2011**). Their applications extend beyond aquaculture to drug delivery, vaccine development, and agriculture, further underscoring their versatility (**Nasr-Eldahan *et al*., 2021**).

Several studies have demonstrated the beneficial effects of dietary chitosan and its Nano form on fish growth, feed utilization, immune response, and survival (**Zaki *et al*., 2015; Akbary & Younesi, 2017; Yan *et al*., 2017; Thilagar & Samuthirapandian, 2020)**. **Niu *et al*. (2011)** identified chitosan as an active growth promoter. On the other hand, **Qi *et al*. (2004)** reported that chitosan nanoparticles exhibit enhanced antimicrobial properties compared with larger chitosan particles. Similarly, **Abd El-Naby *et al*. (2019)** observed improvements in growth performance, body composition, digestive enzyme activities, and immune status of the Nile tilapia fed diets containing 3– 5g/ kg CsNPs. **Abdel-Ghany and Salem (2020)** also found that supplementing 2.5g/ kg chitosan improved survival rates in *Oncorhynchus mykiss* under stress conditions. Furthermore, **Niu *et al.* (2011)** reported that the optimal dietary chitosan inclusion for *Litopenaeus vannamei* ranges between 2.13 and 2.67g/ kg, emphasizing the importance of precise dosage in achieving maximum benefits.

In line with these findings, **Hossam-Elden *et al*. (2024)** highlighted the growing interest in chitosan as a feed additive and emphasized the need for further studies to explore its Nano form for enhanced efficacy. Accordingly, the present study is designed to investigate the effects of dietary Nano-chitosan supplementation on the growth performance, feed utilization, whole-body composition, energy retention, protein productive value (PPV%), and economic efficiency of the Nile tilapia (*O. niloticus*) fingerlings.

MATERIALS AND METHODS

Experimental design and objectives

This study was conducted to evaluate the effects of incorporating dietary Nano-chitosan at different inclusion levels in the diets of the Nile tilapia (*Oreochromis niloticus*) fingerlings on their growth performance, feed utilization, whole-body composition, energy retention (%), protein productive value (PPV%), and economic efficiency.

Experimental site

The experimental study was operated at the Fish Experimental Laboratory belonging to the Animal Production Department, Biological Agriculture Research Institute, National Research Centre, 33 El-Bohouth Street, P.O. Box: 12622, Dokki, Cairo, Egypt. Throughout co-operation work with Department of Cell Biology, Biotechnology Research Institute, National Research Centre, Giza, Dokki, Giza, 12622, Egypt and Hydrobiology Department, Veterinary Research Institute, National Research Centre, 33 El-Bohouth Street, P.O:12622, Dokki, Cairo, Egypt.

Preparation of chitosan nanoparticles

Chitosan nanoparticles (CNPs) were prepared using the ionic gelation method with sodium tripolyphosphate (TPP), as described by **Ahmed *et al.* (2021)**. Briefly, chitosan (0.5% w/v) purchased from Sigma-Aldrich (St. Louis, MO, USA) was dissolved in 1% (w/v) acetic acid and magnetically stirred at room temperature for 30min until a clear solution was obtained. A 0.25% (w/v) solution of TPP (Sigma-Aldrich, St. Louis, MO, USA) was then added dropwise to the chitosan solution at a ratio of 1:3 under continuous magnetic stirring for 45min to promote cross-linking between the positively charged amine groups of chitosan and the negatively charged groups of TPP, forming nanoparticles. The resulting suspension was centrifuged at 1100 × g for 20min, and the supernatant was discarded. The precipitate was washed with distilled water, centrifuged again for purification, and finally lyophilized to obtain dry chitosan nanoparticles suitable for storage and experimental use.

Experimental fish and rearing conditions

A total of 168 the Nile tilapia (*Oreochromis niloticus*) fingerlings, with an initial average weight of 102 ± 0.48g per aquarium, were obtained from Abbassa Fish Hatchery, Sharkia Governorate, Egypt. Fish were acclimated for two weeks in glass aquaria (80 × 40 × 30cm) containing 60L of dechlorinated tap water and fed a control diet during this period. After acclimation, the fish were randomly distributed into 12 aquaria (14 fish per aquarium) to establish the experimental units.

Experimental design and diets

Four experimental diets were prepared to evaluate the effects of Nano-chitosan supplementation on fish performance. A basal control diet was formulated to meet the nutritional requirements of the species, and three additional diets were produced by incorporating nano-curcumin at 2, 4, and 6 mg kg^{-1} feed, respectively. All dry ingredients (Table 1) were finely ground to pass a 500µm sieve and thoroughly mixed in a mechanical blender for 5min. Fish oil and approximately 30– 35 % water were gradually added to obtain a homogeneous dough. For the supplemented diets, the required amount of nano-curcumin powder was first premixed with a small portion of the basal feed to ensure uniform distribution, then blended into the full batch. The dough was pelleted using a laboratory pelletizer fitted with a 2mm die, producing pellets approximately 2–3mm long. Pellets were dried in a forced-air oven at 55°C until moisture content was below 10%, then cooled to room temperature. After drying, pellets were stored in airtight, light-proof polyethylene bags at 4°C until use; representative samples from each diet were analyzed for proximate composition (moisture, crude protein, lipid, ash, and fiber) following **AOAC (2019)** procedures. Nano-curcumin inclusion levels were verified by weighing accuracy during mixing, and all diets were prepared from the same basal mixture to ensure comparable nutrient profiles except for Nano-chitosan concentration.
The fish were randomly assigned to four experimental groups:

G1 (Control): Fed basal diet without Nano-chitosan.

G2: Fed basal diet supplemented with 2g Nano-chitosan/kg diet.

G3: Fed basal diet supplemented with 4g Nano-chitosan/kg diet.

G4: Fed basal diet supplemented with 6g Nano-chitosan/kg diet.

The feeding trial lasted for 56 days, from August 1 to September 30, 2024. The composition of the experimental diets is presented in Table (1).

Table 1. Composition of the different experimental diets

Item	**Experimental diets**				**Price of tone LE**
	Zero Nano chitosan	**2 g Nano-chitosan / kg diet**	**4 g Nano-chitosan / kg diet**	**6 g Nano-chitosan / kg diet**	
	D_1	**D_2**	**D_3**	**D_4**	
Composition of tested diets					
	Basal diet	**Tested diets**			
Soybean meal (44%)	**40**	**Basal diet + 2 g Nano-chitosan / kg diet**	**Basal diet + 4 g Nano-chitosan / kg diet**	**Basal diet + 6 g Nano-chitosan / kg diet**	**30000**
Protein concentration (56%)	**17**				**19000**
Ground Yellow corn (8%)	**28**				**11500**
Wheat bran (13%)	**10**				**9100**
Vegetable oil	**3**				**50000**
Vitamin and Minerals mixture*	**2**				**50000**
Price of ton fed (LE)	**21860**	**21910**	**21960**	**22010**	**----**
Price of kg fed (LE)	**21.860**	**21.910**	**21.960**	**22.010**	**----**

*Vitamin and Minerals mixture: contained Vit. A (E672) (IU) 876.19, Vit. D3 (IU) 1141.39, Vit. E 114.30, Vit. K3 7.55, Vit. B1 13.71, Vit. B2 11.44, Vit. B6 15.33, Vit. B12 0.03, Niacin 60.96, Calpan 30.48, Folic Acid 3.04, Biotin 0.37, Vit. C 11.44, Selenium 0.27, Manganese 19.04, Iron 9.15, Iodine 0.77, Zinc 76.19, Copper 3.04, Cobalt 0.37, Choline Chloride 457.14, and Antioxidant 95.23 (Vit. vitamin; IU international unit).
Price of ton in LE according to 2024.

Parameters of growth performance (NRC, 2011)

Body weight gain (BWG) = Final weight - Initial weight.

Survival rate (SR %) = Number of fish at final / Number of fish at start x100.

Specific growth rate (SGR) =

[In final weight (g) - In initial weight (g)] / Experimental days *100

Calculation of feed conversion ratio (FCR)

FCR = total dry matter intake, (TDMI), g / total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = total body weight gain (TBWG), g / total crude protein intake (TCPI), g.

Feed efficiency

Feed efficiency (FE %) = [weight gain (g) / feed intake (g)]

Protein productive value (PPV %) = [PR_1– PR_0 / PI] 100.

Where: PR_1 = is the total fish body protein at the end of the experiment.

PR_0 = is the total fish body protein at the start of the experiment.

PI = Protein intake.

Energy retention percentages (ER %)

The energy retention percentage was calculated according the following equation:

Energy retention (ER %) = $E\text{-}E_0$ / E_F X 100

Where: E= the energy in fish carcass (kcal) at the end of the experiment.

E_0= the energy in fish carcass (kcal) at the start of the experiment.

E_F = the energy (kcal) in feed intake.

Body composition

At the start of the experiment, 10 fish were randomly sampled to determine baseline whole-body composition. At the end of the feeding trial, seven fish from each treatment group were randomly selected, euthanized, and analyzed to determine whole-body composition.

Economic efficiency of diets

The economic efficiency of the experimental diets was evaluated using an input-output approach as follows:

Feed cost per kg weight gain = Feed conversion ratio × Cost of 1 kg diet

Profit per kg weight gain = Sale price of 1 kg weight gain − Feed cost per kg weight gain

Economic efficiency = Profit per kg weight gain ÷ Feed cost per kg weight gain

Analytical procedures

Proximate composition of the experimental diets and fish body samples was determined following **AOAC (2016)** methods.

Calculated data

The gross energy (kcal/kg DM) of diets and fish body composition was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**, using the following conversion factors: protein (CP) = 5.65 kcal/g, ether extract (EE) = 9.40 kcal/g, crude fiber (CF) and nitrogen-free extract (NFE) = 4.15 kcal/g.

Metabolizable energy (ME) of the diets was calculated according to **NRC (2011)** using 4.50, 8.15, and 3.49 kcal/g for protein, fat, and carbohydrate, respectively. Protein-energy ratio (mg CP/kcal ME) was also calculated as described by **NRC (2011)**.

Statistical analysis

All collected data were analyzed using one-way analysis of variance (ANOVA) in **SPSS (version 2020)**. Differences between treatment means were further assessed using Duncan's Multiple Range Test (**Duncan, 1955**).

RESULTS

Chemical composition of experimental diets

The proximate analysis of the experimental diets is presented in Table (2). Crude protein (CP) content was relatively consistent across the four diets, ranging from 30.50 to 30.57%. Ether extract (EE) values varied slightly between 3.85 and 3.90%. Gross energy of the diets ranged from 4503 to 4507kcal/ kg DM, indicating that all diets were formulated to meet the nutritional requirements of the Nile tilapia. Overall, the experimental diets were iso-caloric and iso-nitrogenous.

Metabolizable energy (ME) values ranged from 353.91 to 354.19kcal/ kg DM, while the protein-to-energy ratio (mg CP/kcal ME) ranged from 86.11 to 86.36, confirming that the diets provided balanced energy relative to protein content.

These results suggest that all experimental diets were nutritionally adequate for supporting the growth and physiological needs of the Nile tilapia fingerlings.

Table 2. Chemical analysis of different experimental diets

Item	Experimental diets			
	Zero Nano chitosan	2 g Nano-chitosan / kg diet	4 g Nano-chitosan / kg diet	6 g Nano-chitosan / kg diet
	D_1	D_2	D_3	D_4
Moisture	7.20	7.18	7.19	7.16
Dry matter (DM)	92.80	92.82	92.81	92.84
Chemical analysis on DM basis				
Organic matter (OM)	92.65	92.62	92.60	92.61
Crude protein (CP)	30.50	30.55	30.57	30.53
Crude fiber (CF)	5.20	5.19	5.17	5.18
Ether extract (EE)	3.90	3.88	3.86	3.85
Nitrogen free extract (NFE)	53.05	53.00	53.00	53.05
Ash	7.35	7.38	7.40	7.39
Energetic values				
Gross energy kcal/ kg DM	4507	4506	4504	4503
Gross energy cal/ g DM	4.507	4.506	4.504	4.503
Metabolizable energy kcal/ kg DM	354.18	354.07	353.99	353.91
Protein energy ratio (mg CP/ Kcal ME)	86.11	86.24	86.36	86.26

Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

Metabolizable energy (ME): Calculated according to **NRC (2011)** using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively.

Growth performance and survival

The effects of dietary Nano-chitosan supplementation on growth performance and survival of the Nile tilapia fingerlings are presented in Table (3).

Inclusion of 2g/ kg Nano-chitosan (G2) in the diet resulted in a significant (*P*< 0.05) improvement in final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) compared with the other groups (G1, G3, and G4). Among the treatments, G2 recorded the highest growth values, followed by G3 and G1, while G4 exhibited the lowest significant (*P*< 0.05) growth performance.Survival rates were 100% in G1, G2, and G3, whereas G4 showed a markedly lower survival rate of 71.43%. Correspondingly, the mortality rate was 0% in G1, G2, and G3 but reached 28.57% in G4.

These results indicate that low-level dietary Nano-chitosan (2 g/kg) enhances growth performance and maintains high survival in the Nile tilapia fingerlings, whereas higher inclusion levels (6g/ kg) may negatively affect survival.

Table 3. Growth performance, specific growth rate and survival ratio of the Nile tilapia fingerlings fed diets containing different levels of dietary Nano-chitosan

Item	Experimental groups				SEM	Sign. *P*<0.05
	Zero Nano chitosan	2 g Nano-chitosan / kg diet	4 g Nano-chitosan / kg diet	6 g Nano-chitosan / kg diet		
	G_1	G_2	G_3	G_4		
Number of fish	42	42	42	42	-	-
IW, g	101	103	102	102	0.477	NS
FW, g	426^b	505^a	428^b	357^c	15.886	*
TBWG, g	325^b	402^a	326^b	255^c	15.886	*
Duration trial	56 days					
ADG, g	5.80^b	7.18^a	5.82^b	4.55^c	0.284	*
SGR	1.12^b	1.23^a	1.12^b	0.97^c	0.029	*
RGR	3.20^b	3.90^a	3.20^b	2.50^c	0.152	*
Starter number	42	42	42	42	-	-
End number of	42	42	42	30	-	-
SR %	100	100	100	71.43	-	-
Dead number	Zero	Zero	Zero	12	-	-
Mortality rate %	Zero	Zero	Zero	28.57	-	-

a, b and c: Means in the same row having different superscripts differ significantly (*P*<0.05).

Feed utilization of the different experimental groups

The effects of dietary Nano-chitosan on feed utilization parameters are presented in Table (4). Fish fed the diet supplemented with 2g/ kg Nano-chitosan (G2) showed a significant (*P*< 0.05) increase in feed intake (FI) and crude protein intake (CPI) compared to the other groups (G1, G3, and G4). Specifically, G2 recorded the highest FI (511g) and CPI (156.11g), while values in the other groups were as follows: G1 — FI 443g and CPI 135.12g; G3 — FI 445g and CPI 136.04g; G4 — FI 386g and CPI 117.85g. Feed conversion ratio (FCR) was significantly improved in G2, with the lowest FCR value of 1.271, indicating more efficient feed utilization. The FCR values for G1, G3, and G4 were 1.363, 1.365, and 1.514, respectively. Similarly, protein efficiency ratio (PER) was highest in G2 (2.575), followed by G1 (2.405), G3 (2.396), and G4 (2.164).

Table 4. Feed utilization of the Nile tilapia fingerlings fed diets contained different levels of dietary Nano-chitosan

Item	Experimental groups				SEM	Sign. P<0.05
	Zero Nano chitosan	2 g Nano-chitosan / kg diet	4 g Nano-chitosan / kg diet	6 g Nano-chitosan / kg diet		
	G_1	G_2	G_3	G_4		
TBWG, g	**325^{b}**	**402^{a}**	**326^{b}**	**255^{c}**	**15.886**	*
FI, g	**443^{b}**	**511^{a}**	**445^{b}**	**386^{c}**	**13.445**	*
FCR	**1.363^{a}**	**1.271^{b}**	**1.365^{b}**	**1.514^{c}**	**0.027**	*
FCP%	**30.50**	**30.55**	**30.57**	**30.53**	-	-
CPI, g	**135.12^{b}**	**156.11^{a}**	**136.04^{b}**	**117.85^{c}**	**4.116**	*
PER	**2.405^{b}**	**2.575^{a}**	**2.396^{b}**	**2.164^{c}**	**0.045**	*

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).

SEM: Standard error of the mean, *: Significant at (P<0.05), FI: Feed intake, TBWG: Total body weight gain, FCR: Feed conversion ratio, FCP%: Feed crude protein percentages, CPI: Crude protein intake, PER: Protein efficiency ratio.

Fish body composition of different experimental group

The effects of dietary Nano-chitosan on the whole-body composition of the Nile tilapia are presented in Table (5). Incorporation of Nano-chitosan at 2, 4, and 6g/ kg diet (G2, G3, and G4) significantly (P < 0.05) reduced dry matter (DM) content compared with the control group (G1), while moisture content significantly increased.

Regarding organic matter (OM), the highest value was observed in G3 (85.74%), followed by G4 (85.67%) and G2 (82.80%), whereas G1 had the lowest OM content (82.78%). Ash content was highest in the control group (G1, 17.22%), followed closely by G2 (17.20%), while G3 and G4 showed lower ash levels (14.26% and 14.33%, respectively).

Crude protein (CP) content was significantly enhanced by Nano-chitosan supplementation, with the highest values in G3 (68.21%), followed by G4 (68.00%) and G2 (65.10%), while G1 had the lowest CP content (62.58%). In contrast, ether extract (EE) was significantly reduced in G2, G3, and G4 compared with the control.

Gross energy (kcal/100 g DM) was significantly higher in G4 (550.30 kcal/ 100g DM), followed by G3 (550.17 kcal/ 100g DM), whereas G2 recorded the lowest value (534.20 kcal/ 100g DM).

These results indicate that dietary Nano-chitosan modulates body composition by increasing protein content and energy values while reducing fat and ash contents in the Nile tilapia fingerlings.

Table 5. Fish body composition of initial and different experimental groups of the Nile tilapia fingerlings fed diets containing different levels of dietary Nano-chitosan

Item	Fish body composition of initial fish	Experimental diets				SEM	Sign. $P<0.05$
		Zero Nano chitosan	2 g Nano-chitosan / kg diet	4 g Nano-chitosan / kg diet	6 g Nano-chitosan / kg diet		
		G_1	G_2	G_3	G_4		
Moisture	**76.64**	**74.22^d**	**77.52^a**	**75.70^b**	**75.00^c**	**0.369**	*
DM	**23.36**	**25.78^a**	**22.48^d**	**24.30^c**	**25.00^b**	**0.369**	*
Chemical analysis on DM basis		**Chemical analysis on DM basis**				-	-
OM	**85.18**	**82.78^b**	**82.80^b**	**85.74^a**	**85.67^a**	**0.440**	*
CP	**61.24**	**62.58^d**	**65.10^c**	**68.21^a**	**68.00^b**	**0.697**	*
EE	**23.94**	**20.20^a**	**17.70^b**	**17.53^b**	**17.67^b**	**0.337**	*
Ash	**14.82**	**17.22^a**	**17.20^a**	**14.26^b**	**14.33^b**	**0.440**	*
GE1	**571.04**	**543.46^b**	**534.20^c**	**550.17^a**	**550.30^a**	**1.992**	*
GE2	**5.7104**	**5.4346^b**	**5.3420^c**	**5.5017^a**	**5.5030^a**	**0.020**	*

a, b, c and d: Means in the same row having different superscripts differ significantly ($P<0.05$).

SEM: Standard error of the mean.

*: Significant at $P<0.05$, DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, GE1: Gross energy kcal/ 100g, GE2: Gross energy Cal/ g DM

Energy retention and protein productive value percentages of different experimental groups

The effects of dietary Nano-chitosan on energy retention (%) in the Nile tilapia fingerlings are presented in Table (6). The highest energy retention (91.62%) was observed in fish fed the diet containing 2g/ kg Nano-chitosan (G2). The other groups recorded lower values, with 87.03%, 88.47%, and 79.57% for G1, G3, and G4, respectively.

Similarly, the net energy retention was greatest in G2, reaching 170.19%, while the remaining groups showed values of 151.52%, 168.69%, and 152.99% for G1, G3, and G4, respectively.

These results indicate that dietary inclusion of 2g/ kg Nano-chitosan optimizes energy retention in the Nile tilapia fingerlings, supporting more efficient utilization of feed nutrients.

Economical evaluation of different experimental groups

The economic impact of dietary Nano-chitosan supplementation is presented in Table (7). Incorporation of Nano-chitosan slightly increased the cost of feed formulation, from 21.860 LE/kg for the control diet (G1) to 21.910, 21.960, and 22.010 LE/kg for G2, G3, and G4, respectively.

The highest improvement in net feed cost efficiency (%) was observed in G2, where supplementation with 2g/ kg Nano-chitosan increased economic efficiency by

6.76% compared with the control (G1, considered as zero). In contrast, G3 and G4 showed negative improvements of −0.14% and −11.12%, respectively.

These results indicate that under the present experimental conditions, dietary inclusion of 2g/ kg Nano-chitosan is optimal for improving economic efficiency, whereas higher inclusion levels (4– 6g/ kg) are less cost-effective and may reduce profitability.

Table 6. Energy retention (ER) and protein productive value (PPV) %of the Nile tilapia fingerlings fed diets containing different levels of dietary Nano-chitosan

Item	**Experimental diets**				**SEM**	**Sign. *P*<0.05**
	Zero Nano chitosan	**2 g Nano-chitosan / kg diet**	**4 g Nano-chitosan / kg diet**	**6 g Nano-chitosan / kg diet**		
	G_1	**G_2**	**G_3**	**G_4**		
IW	**101**	**103**	**102**	**102**	**0.477**	**NS**
FW	**426^{b}**	**505^{a}**	**428^{b}**	**357^{c}**	**15.886**	*****
Calculation of the energy retention						
ECFBW	**5.4346^{b}**	**5.3420^{c}**	**5.5017^{a}**	**5.5030^{a}**	**0.020**	*****
TEEBF	**2315^{b}**	**2698^{a}**	**2355^{b}**	**1965^{c}**	**79.209**	*****
ECIBF	**5.7104**				**-**	**-**
TESBF	**577**	**588**	**582**	**582**	**2.694**	**NS**
ERBF	**1738^{b}**	**2110^{a}**	**1773^{b}**	**1383^{c}**	**78.895**	*****
EFI	**4.507**	**4.506**	**4.504**	**4.503**	-	-
QFI	**443^{b}**	**511^{a}**	**445^{b}**	**386^{c}**	**13.445**	*****
TEFI	**1997^{b}**	**2303^{a}**	**2004^{b}**	**1738^{c}**	**60.763**	*
ER%	**87.03^{b}**	**91.62^{a}**	**88.47^{b}**	**79.57^{c}**	**1.391**	*
Calculation of the protein productive value (PPV) %						
CPFBC%	**62.58^{d}**	**65.10^{c}**	**68.21^{a}**	**68.00^{b}**	**0.697**	*****
PR_1	**266.59^{c}**	**328.76^{a}**	**291.94^{b}**	**242.76^{d}**	**9.721**	*****
CPIBFC%	**61.24**				**-**	**-**
PR_2	**61.85**	**63.08**	**62.46**	**62.46**	**0.292**	**NS**
PR_3	**204.74^{c}**	**265.68^{a}**	**229.48^{b}**	**180.30^{d}**	**9.624**	*****
CPFE%	**30.50**	**30.55**	**30.57**	**30.53**	-	-
TPI, g	**135.12^{b}**	**156.11^{a}**	**136.04^{b}**	**117.85^{c}**	**4.116**	*****
PPV%	**151.52^{b}**	**170.19^{a}**	**168.69^{a}**	**152.99^{b}**	**2.680**	*****

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).
SEM: Standard error of the mean, NS: Not significant, *: Significant at (P<0.05), IW: Initial weight, g, FW: Final weight, g, ECFBW: Energy content in final body fish (cal / g),TEEBF: Total energy at the end in body fish (E), Energy content in initial body fish (cal / g), TESBF: Total energy at the start in body fish (E_0), Energy retained in body fish ($E-E_0$), EFI: Energy of the feed intake (Cal / g feed), QFI: Quantity of feed intak, TEFI: Total energy of feed intake (EF). ER%: Energy retention (ER) %, CPFBC%: Crude protein % in final body fish, PR_1: Total protein at the end in body fish, CPIBFC%: Crude protein % in initial body fish, PR_2: Total protein at the start in body fish, PR3: Protein Energy retained in body fish (PR_3) = (PR_1 – PR_2), CPFI: Crude protein in feed intake (CP %),TPI: Total protein intake(g) , PPV%: Protein productive value.

*

Table 7. Economical evaluation of the Nile tilapia fingerlings fed diets containing different levels of dietary Nano-chitosan

Item	**Experimental diets**			
	Zero Nano chitosan	**2 g Nano-chitosan / kg diet**	**4 g Nano-chitosan / kg diet**	**6 g Nano-chitosan / kg diet**
	G_1	**G_2**	**G_3**	**G_4**
Costing of kg feed (LE)	**21.860**	**21.910**	**21.960**	**22.010**
Relative to control (%)	**100**	**100.22**	**100.46**	**100.69**
Feed conversion ratio (FCR)	**1.363**	**1.271**	**1.365**	**1.514**
Feeding cost (LE) per (Kg weight gain)	**29.80**	**27.85**	**29.98**	**33.32**
Relative to control (%)	**100**	**93.46**	**100.60**	**111.81**
Net improving in feeding cost (%)	**Zero**	**6.76**	**- 0.14**	**- 11.12**

Diet formulation calculated according to the local prices at year 2024, as presented in Table (1).
Feed cost (L.E) FCR×FI. Cost per Kg diet.

DISCUSSION

The chemical analysis of the experimental diets indicated that crude protein (CP) ranged from 30.50 to 30.57%, while ether extract (EE) ranged from 3.85 to 3.90%. Gross energy varied from 4503 to 4507 kcal/kg DM, metabolizable energy (ME) ranged from 353.91 to 354.19 kcal/kg DM, and protein energy ratio (PER) ranged from 86.11 to 86.36mg CP/Kcal ME. These values are considered adequate to meet the Nile tilapia nutritional requirements, and the diets were iso-caloric and iso-nitrogenous. These findings are consistent with those reported by **Abozaid *et al*. (2024a)**, who observed similar ranges for CP (30.15–30.80%), gross energy (4543–4559 kcal/kg), ME (351.37–353.94 kcal/kg DM), and PER (85.18–87.66 mg CP/Kcal ME).

In terms of growth performance, the supplementation of 2g/ kg Nano-chitosan (G2) significantly improved final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) compared to the other groups. Survival was 100% in G1, G2, and G3, but decreased to 71.43% in G4, with a corresponding mortality rate of 28.57%. These results align with previous studies demonstrating the growth-promoting and immunomodulatory effects of chitosan and chitosan nanoparticles (CNPs) in the Nile tilapia. For instance, **Hossam-Elden *et al.* (2024)** observed significant improvements in growth metrics with 1– 3g CNPs/kg diet, while higher concentrations (5g/ kg) were less effective. Similarly, **Aboseif *et al*. (2024)** reported that 0.1– 0.2g CsNPs/kg diet enhanced growth performance, SGR, energy efficiency ratio (EER), and survival, while higher levels did not provide additional benefits.

The enhanced growth performance may be attributed to the ability of Nano-chitosan to improve nutrient absorption, modulate gut microbiota, and increase the height of intestinal villi (**Abd El-Naby *et al*., 2019; Poznanski *et al*., 2023**). Chitosan nanoparticles also exhibit higher surface-to-charge density, larger surface area, and better cellular uptake compared to bulk chitosan, enhancing their biological activity. Moreover, chitosan can promote immune function and reduce the impact of pathogens, contributing to overall fish health and performance (**Assadpour *et al*., 2016; Fadl *et al*., 2020)**.

Feed utilization parameters further support the growth results. Group G2 (2 g/kg Nano-chitosan) showed the highest feed intake (FI = 511 g), crude protein intake (CPI = 156.11 g), protein efficiency ratio (PER = 2.575), and the lowest feed conversion ratio (FCR = 1.271). These findings are consistent with **Kamali *et al*. (2016)** and **Dawood *et al*. (2020)**, who reported improvements in FCR and PER with dietary chitosan supplementation. However, excessive inclusion (G4, 6g/ kg) negatively affected feed utilization, likely due to reduced digestibility and nutrient absorption caused by high chitosan levels (**Shiau & Yu, 1999**).

Fish body composition was also affected by Nano-chitosan supplementation. Dry matter (DM) decreased while moisture content increased in all treated groups. Organic matter (OM) and crude protein (CP) were the highest in G3 (4g/ kg) and G4 (6g/ kg), while ether extract (EE) decreased in all treated groups. Gross energy was at its highest value in G4, suggesting that higher inclusion may promote energy retention but may not correspond to improved growth performance. These effects are in agreement with **Abu-Zahra *et al*. (2025)**, who observed increased protein content and decreased lipids in *O. niloticus* fed chitosan-supplemented diets. The observed changes may result from improved nutrient absorption, enhanced gut health, and altered lipid metabolism (**Shiau & Yu, 1999; Yan *et al*., 2017**).

Energy retention (ER%) and protein productive value (PPV%) were the highest in G2 (91.62% and 170.19%, respectively), indicating optimal nutrient utilization at 2g/ kg Nano-chitosan. These findings are consistent with studies on nano-supplements, such as *Saccharomyces cerevisiae* (**Abozaid *et al*., 2024a, b**) and Nano-curcumin (**Abozaid *et al*., 2025b**), which also reported enhanced ER% and PPV% at moderate inclusion levels.

Economically, the inclusion of Nano-chitosan increased feed costs slightly (21.860 LE/kg in G1 to 22.010 LE/kg in G4), but net improvement in feeding efficiency was the highest in G2 (+6.76%), whereas G3 and G4 showed negative improvements. These results indicate that 2g/ kg Nano-chitosan is economically optimal under the experimental conditions. Similar observations were reported with other feed additives, including the black soldier fly meal (**Hebicha *et al*., 2013; Fayed *et al*., 2023**), *Galleria mellonella* larvae (**Abozaid *et al*., 2024c**), dry yeast (**Abozaid *et al*., 2025a**), and Nano-curcumin (**Abozaid *et al*., 2025b**), where moderate supplementation improved economic efficiency, while excessive inclusion reduced cost-effectiveness.

Overall, the present study demonstrates that dietary Nano-chitosan at 2g/ kg can enhance growth performance, feed utilization, body composition, nutrient retention, and economic efficiency in the Nile tilapia. Higher doses may have detrimental effects on survival, feed utilization, and profitability, highlighting the importance of optimizing supplementation levels in aquaculture diets.

CONCLUSION

The present study demonstrated that dietary supplementation of Nano-chitosan positively influenced growth performance, feed utilization, body composition, energy retention, and economic efficiency in the Nile tilapia (*Oreochromis niloticus*). Among the tested levels, 2g/kg Nano-chitosan was the most effective, significantly improving final weight, weight gain, feed conversion ratio, protein efficiency, energy retention, and survival rate, while maintaining cost-effectiveness. Higher inclusion levels (4– 6g/ kg) did not provide additional benefits and, in some cases, negatively affected growth and feed efficiency. These findings suggest that incorporating 2g/ kg Nano-chitosan into tilapia diets is optimal for enhancing growth performance, nutrient utilization, and profitability in aquaculture operations.

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