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Effects of Dietary Nano Zinc Oxide Supplementation and Rearing Temperature on the Performance and Thermal Resistance of the Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ABSTRACT

A total of 180 Nile tilapia (Oreochromis niloticus) fingerlings were used to assess the effects of dietary nano zinc oxide (N-ZnO) supplementation at 0, 15, and 30mg/ kg feed under two water temperature regimes (28 and 34°C). The experiment comprised six groups: G1 (control, 28°C), G2 (control, 34°C), G3 (15 mg/kg N-ZnO, 28°C), G4 (15 mg/kg N-ZnO, 34°C), G5 (30 mg/kg N-ZnO, 28°C), and G6 (30 mg/kg N-ZnO, 34°C). Fish were randomly distributed across 18 aquaria (10 fish per tank), with an average initial body weight of 154.67 ± 0.478 g. The feeding trial lasted for 75 days, during which groups G2, G4, and G6 were exposed to 34°C for the final 15 days. All diets were formulated to be iso-caloric and iso-nitrogenous. Growth performance parameters including final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) significantly improved with increasing levels of N-ZnO supplementation. Feed intake (FI) and crude protein intake (CPI) also increased. Notable enhancements ($P< 0.05$) were observed in crude protein (CP), ether extract (EE), organic matter (OM), ash content, energy retention (ER%), and protein productive value (PPV%), while moisture, dry matter (DM), and gross energy remained unchanged. Fish receiving 15 or 30mg/ kg N-ZnO exhibited greater resilience to thermal stress, maintaining superior growth and nutrient utilization at 34°C compared to the control. Economically, feed cost per unit of weight gain was reduced by 6.78 to 32.60% in treated groups. In conclusion, dietary N-ZnO enhanced growth, thermal tolerance, nutrient efficiency, and economic returns in the Nile tilapia.

INTRODUCTION

Aquaculture continues to play a pivotal role in addressing the increasing global demand for protein of animal origin. The Nile tilapia (*Oreochromis niloticus*) is widely cultivated due to its fast growth, adaptability to varied environmental conditions, and high nutritional value. Among the key elements determining success in aquaculture systems, nutrition stands out as a crucial factor influencing fish health, growth rates, feed conversion, and overall production efficiency **(Ghaly *et al*., 2024; Mustafa *et al*., 2024)**. In intensive aquaculture operations, however, over-supplementation of minerals in fish diets may result in nutrient build-up in the environment, contributing to issues such as eutrophication. Therefore, it is important to design diets that provide essential minerals in appropriate amounts to meeting the species' physiological needs while avoiding ecological harm from excess discharge.

Zinc is one of the essential trace minerals required in fish diets, since it plays multiple roles in metabolic pathways, enzymatic function, immune regulation, and antioxidant defense mechanisms **(Wang *et al*., 2008; NRC, 2011; Moazenzadeh *et al*., 2017)**. It is also a structural or functional component of numerous enzymes and proteins that maintain cellular health, genetic stability, and resistance to oxidative damage **(Salgueiro *et al*., 2000; Ho & Ames, 2002)**. Despite its importance, zinc in excessive concentrations may become toxic, potentially disrupting physiological functions and posing risks to aquatic life **(Bielmyer *et al*., 2012; NIH, 2013)**.

In recent years, the use of nanotechnology in aquafeeds has gained attention as a strategy to enhance nutrient delivery and improve bioavailability. Nano-sized zinc oxide particles (ZnO NPs) possess unique properties, including increased surface area and solubility, which may lead to improved absorption and biological activity compared to conventional forms **(Khosravi-Katuli *et al*., 2017; Fasil *et al*., 2020)**. Studies have suggested that these features can support better growth, feed efficiency, antioxidant status, and immune responses in fish at reduced supplementation levels **(Ellis *et al*., 2004; Zhou *et al*., 2009; Liu *et al*., 2018)**. However, the physiological effects of ZnO nanoparticles are influenced by factors such as particle size, dosage, and synthesis method and inappropriate use may result in toxicity **(Franklin *et al*., 2007; Lin & Xing, 2007)**.

Water temperature is another major environmental factor affecting fish physiology. Shifts in temperature can alter metabolic demands, stress tolerance, and nutrient processing in aquatic species. Hence, it is important to examine how dietary nano zinc interacts with different temperature conditions to influence fish performance.

In light of these considerations, the current study investigated the impact of dietary inclusion of zinc oxide nanoparticles at varying concentrations on the Nile tilapia reared under different thermal conditions. The research evaluated their effects on growth metrics, nutrient utilization, body composition, gene expression, and feed cost efficiency, aiming to inform sustainable feeding practices under changing environmental conditions.

MATERIALS AND METHODS

This study was conducted to assess the effects of dietary nano zinc oxide (N-ZnO) supplementation on the growth performance, feed efficiency, and economic outcomes of the Nile tilapia (*Oreochromis niloticus*) fingerlings under both standard (28°C) and elevated (34°C) water temperature conditions. The experimental work took place at the Fish Experimental Laboratory, Animal Production Department, Biological Agriculture Research Institute, National Research Centre (NRC), located at 33 El-Bohouth Street, P.O. Box 12622, Dokki, Cairo, Egypt. The research was carried out in collaboration with the Hydrobiology Department of the Veterinary Research Institute and the Cell Biology Department of the Biotechnology Research Institute, both affiliated with the NRC in Cairo.

Experimental unit

A total of 180 Nile tilapia fingerlings were acclimated and then randomly allocated to 18 glass aquaria (each measuring 80 × 40 × 30 cm and filled with 60L of dechlorinated tap water), with 10 fish per tank. The average initial weight per fish was 154.67 ± 0.478g. Experimental diets were prepared by incorporating N-ZnO particles at two levels: 15 mg/kg and 30 mg/kg of feed. Fish were obtained from the Abbassa Fish Hatchery (Sharkia Governorate, Egypt). Prior to the experiment, fish were maintained on a basal diet during a 14-day adaptation period.

Experimental designed and diets

The fish were divided into six treatment groups as follows:

G1: Basal diet, reared at 28 °C (control).

G2: Basal diet, reared at 34 °C.

G3: Basal diet + 15 mg/kg N-ZnO, reared at 28°C.

G4: Basal diet + 15 mg/kg N-ZnO, reared at 34°C.

G5: Basal diet + 30 mg/kg N-ZnO, reared at 28°C.

G6: Basal diet + 30 mg/kg N-ZnO, reared at 34°C.

The feeding trial lasted for 75 days, from early January to mid-March 2024. Fish in the groups exposed to elevated temperatures (G2, G4, and G6) were maintained at 28°C for the initial 60 days, after which the water temperature was gradually increased to 34°C for the final 15 days. The composition of the experimental diets is shown in Table (1).

Table 1. Composition of the different experimental diets

Item	Experimental diets			Price of tone LE
	D_1 Fed to G_1 and G_2	D_2 Fed to G_3 and G_4	D_3 Fed to G_5 and G_6	
Composition of tested diets				
Soybean meal (44%)	40.00	Basal diet + 15 mg N-ZnO particle size / kg feed	Basal diet + 30 mg N-ZnO particle size / kg feed	33000
Protein concentration (56%)	17.00			25000
Yellow corn (8%)	28.00			12500
Wheat bran (13%)	10.00			14500
Vegetable oil	3.00			50000
Salt (sodium chloride)	1.00			5000
Vitamin and Minerals*	1.00			40000
Nano Zinc Oxide particle size	00.00			5 LE per g
Price of ton fed (LE)	24350	24425	24500	----
Price of kg fed (LE)	24.350	24.425	24.500	----

** 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 tone LE According to 2024.

Parameters of growth performance

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-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 analysis

At the beginning of the trial, 10 fish were stocked per aquarium. At the end of the experiment, five fish were randomly selected from each treatment group for whole-body composition analysis.

Analytical procedures

The chemical composition of the experimental diets and the whole-body samples of fish were analyzed following the standard procedures outlined by the Association of Official Analytical Chemists **(AOAC, 2016)**.

Calculated data

The gross energy content (kcal/kg dry matter) of the experimental diets and the whole-body composition of fish in each treatment group was estimated according to the methods described by **Blaxter (1968)** and **MacRae and Lobley (2003)**, using the following caloric values: crude protein (CP) = 5.65 kcal/g, ether extract (EE) = 9.40 kcal/g, and crude fiber (CF) and nitrogen-free extract (NFE) = 4.15 kcal/g.

Metabolizable energy (ME) was calculated based on the guidelines of the NRC (2011), using energy conversion values of 4.50 kcal/g for protein, 8.15 kcal/g for fat, and 3.49 kcal/g for carbohydrates. Additionally, the protein to energy ratio (mg CP/kcal ME) was determined following **NRC (2011)** recommendations.

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) in **SPSS (2020)**. Differences between treatment means were assessed using Duncan's multiple range test **(Duncan, 1955)**, with significance accepted at $P< 0.05$.

RESULTS

As shown in Table (2), the selected ingredients were appropriate for formulating fish diets and are commonly used on a large scale in aquafeed production.

Table 2. Chemical analysis of feed ingredients that used in Basal diet formulation

Item	Feed ingredients			
	Soybean meal	**Yellow corn**	**Wheat bran**	**Protein Concentration**
Moisture	9.50	9.77	9.96	3.05
Dry matter (DM)	90.50	90.23	90.04	96.95
Chemical analysis on DM basis				
Organic matter (OM)	93.39	98.34	94.64	93.22
Crude protein (CP)	44.00	8.00	13.00	56.00
Crude fiber (CF)	3.69	2.48	8.56	2.84
Ether extract (EE)	2.83	3.75	3.81	1.55
Nitrogen free extract (NFE)	42.87	84.11	69.27	32.93
Ash	6.61	1.66	5.36	6.68
Gross energy kcal/ kg DM	4684	4398	4323	4794
Gross energy cal/ g DM	4.684	4.398	4.323	4.794
Metabolizable energy kcal/ kg DM	370.68	360.11	331.30	379.56
Protein energy ratio (mg CP/ Kcal ME)	118.70	22.22	39.24	147.54

Gross energy (kcal/ kg DM) was calculated according to **Blaxter** (**2017)** and **MacRae and Lobley (2018)**.

Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

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

Chemical analysis of the experimental diets

As shown in Table (3), the crude protein content of the formulated diets ranged from 30.65 to 30.66%. Gross energy was consistent across all treatments at approximately 4652 kcal/kg. Metabolizable energy (ME) values were also uniform, ranging from 372.31 to 372.36 kcal/kg. The protein-to-energy ratio (mg CP/kcal ME) varied slightly between 82.30 and 82.34. These nutrient levels are considered sufficient to fulfill the dietary requirements of the Nile tilapia. All diets were designed to be both iso-caloric and iso-nitrogenous, ensuring comparability across treatments.

Table 3. Chemical analysis of different experimental diets

Item	Experimental diets		
	D_1 Fed to G_1 and G_2	**D_2 Fed to G_3 and G_4**	**D_3 Fed to G_5 and G_6**
Moisture	11.12	11.12	11.14
Dry matter (DM)	88.88	88.88	88.86
Chemical analysis on DM basis			
Organic matter (OM)	93.66	93.65	93.65
Crude protein (CP)	30.66	30.65	30.64
Crude fiber (CF)	3.61	3.61	3.61
Ether extract (EE)	5.82	5.82	5.82
Nitrogen free extract (NFE)	53.57	53.57	53.58
Ash	6.34	6.35	6.35
Gross energy kcal/ kg DM	4652	4652	4652
Gross energy cal/ g DM	4.652	4.652	4.652
Metabolizable energy kcal/ kg DM	372.36	372.32	372.31
Protein energy ratio (mg CP/ Kcal ME)	82.34	82.32	82.30

Gross energy (kcal/ kg DM) was calculated according to **Blaxter (2017)** and **MacRae and Lobley (2018)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.Metabolizable energy (ME):
Calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively. Calculated according to **NRC [2011]**.

Growth and survival ratio

As shown in Table (4), incorporating nano zinc oxide (N-ZnO) particles into the diet significantly enhanced final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) in fish supplemented with either 15 or 30 mg/kg N-ZnO and reared at both 28 and 34°C, compared to the control groups receiving unsupplemented diets at the same respective temperatures. Notably, group D5 (30 mg/kg N-ZnO, 28°C) achieved the highest values across all growth performance parameters, outperforming both control groups (D1 and D2) and the other experimental groups (D3, D4, and D6). In addition, the mortality rate remained at zero across all treatment groups.

Table 4. Growth performance, Specific growth rate and survival ratio of the Nile tilapia *(O. niloticus)* fed diets containing different concentrations of nanozinc and reared in different water temperatures

Item	Experimental groups						SEM	Sign. *P*<0.05
	Fish fed basal diet and reared in water normal temperature (28°) (control No. 1)	Fish fed basal diet and reared in water temperature (34°) (control No. 2)	Fish fed basal diet and 15 mg N-Zn O particle size and reared in normal water temperature (28°)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in water temperature (34°)	Fish fed basal diet and 30 mg N-Zn O particle size and reared in water normal temperature (28°)	Fish fed basal diet and 30 mg N-Zn O particle size and reared in water temperature 34°)		
	G_1	G_2	G_3	G_4	G_5	G_6		
Number of fish	30	30	30	30	30	30	-	-
IW, g	155	154	154	155	156	154	0.478	NS
FW, g	380^d	358^e	552^c	547^c	592^a	580^b	23.09	*
TBWG, g	225^d	204^e	398^c	392^c	436^a	426^b	23.06	*
Duration experimental	**75 days**							
ADG, g	3.00^d	2.72^e	5.31^c	5.23^c	5.81^a	5.68^b	0.307	*
SGR	0.71^c	0.65^d	0.99^b	0.98^b	1.04^a	1.03^a	0.038	*
RGR	1.51^c	1.31^c	2.55^b	2.56^b	2.80^a	2.75^a	0.146	*
Starter number	30	30	30	30	30	30	-	-
End number of	30	30	30	30	30	30	-	-
SR %	100	100	100	100	100	100	-	-
Dead number	Zero	Zero	Zero	Zero	Zero	Zero	-	-
Mortality rate%	Zero	Zero	Zero	Zero	Zero	Zero	-	-

a, b, c, d and e: 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.
TBWG: Total body weight gain, g. Average daily gain, g (ADG), SGR: Specific growth rate. RGR: Relative growth rate, SR: Survival ratio

Feed utilization of the different experimental groups

As presented in Table (5), feed utilization parameters demonstrated a clear upward trend with increasing levels of nano zinc oxide (N-ZnO) supplementation. Feed intake (FI) and crude protein intake (CPI) increased progressively from the control groups (D1 and D2) to the 15 mg/kg N-ZnO groups (D3 and D4), and further in the 30 mg/kg N-ZnO groups (D5 and D6). The highest FI and CPI values were observed in group D5, reaching 736.68 g and 225.72 g, respectively. Moreover, the feed conversion ratio (FCR) significantly improved (*P*< 0.05) in fish receiving diets with 15 or 30mg/ kg N-ZnO compared to the control groups. Similarly, the protein efficiency ratio (PER) increased significantly (*P*< 0.05) with higher levels of N-ZnO, with group D5 (30 mg/kg N-ZnO at 28°C) showing the most favorable FCR and PER values.

Table 5. Feed utilization of the Nile tilapia *(O. niloticus)* fed diets containing different concentrations of Nano zinc and reared in different water temperatures

	Experimental groups							
	Fish fed basal diet and reared in water normal temperature (28°) (control No. 1)	Fish fed basal diet and reared in water temperature (34°) (control No. 2)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in normal water temperature (28°)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in water temperature (34°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water normal temperature (28°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water temperature (34°)	SEM	Sign. *P*<0.05
Item	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**	**G_6**		
TBWG, g	225^{d}	204^{e}	398^{c}	392^{c}	436^{a}	426^{b}	23.06	*
FI, g	561.96	544.74	700.14	698.88	736.68	732.48	37.928	NS
FCR	2.50^{c}	2.67^{d}	1.76^{b}	1.78^{b}	1.69^{a}	1.72^{a}	0.098	*
FCP%	30.66		30.65		30.64		-	-
CPI, g	172.30^{c}	167.02^{d}	214.59^{b}	214.21^{b}	225.72^{a}	224.43^{a}	5.838	*
PER	1.306^{c}	1.221^{d}	1.855^{b}	1.830^{b}	1.932^{a}	1.898^{a}	0.071	*

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),TBWG: Total body weight gain, FI: Feed intake, FCR: Feed conversion ratio, FCP%: Feed crude protein percentages, CPI: Crude protein intake, PER: Protein efficiency ratio

Fish body composition of different experimental groups

The results in Table (6) indicate that dietary inclusion of nano zinc oxide (N-ZnO) particles did not significantly (*P*> 0.05) affect moisture content, dry matter (DM), or gross energy values in the Nile tilapia. However, significant differences (*P*< 0.05) were observed in other compositional parameters, including organic matter (OM), crude protein (CP), ether extract (EE), and ash content. Specifically, supplementation with N-ZnO resulted in a marked decrease in OM and EE levels compared to the control groups. In contrast, the crude protein and ash contents increased significantly (*P*< 0.05) in response to N-ZnO supplementation. Although downward trends were noted for dry matter, OM, EE, and gross energy, these changes were not statistically significant in all instances.

Table 6. Fish body composition of initial and different experimental groups that fed tested diets

	Fish body composition of initial fish	Experimental groups							
		Fish fed basal diet and reared in water normal temperature (28°) (control No. 1)	Fish fed basal diet and reared in water temperature (34°) (control No. 2)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in normal water temperature (28°)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in water temperature (34°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water normal temperature (28°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water temperature 34°)	SEM	Sign. *P*<0.05
Item		**G_1**	**G_2**	**G_3**	**G_4**	**G_5**	**G_6**		
Moisture	70.99	71.59	71.65	71.68	71.69	71.74	71.60	0.023	NS
DM	29.01	28.41	28.35	28.32	28.31	28.26	28.40	0.023	NS
Chemical analysis on DM basis									
OM	82.25	85.23^{a}	84.03^{b}	82.14^{c}	81.16^{d}	82.50^{c}	84.60^{ab}	0.361	*
CP	53.61	55.65^{e}	59.79^{d}	62.05^{ab}	60.65^{c}	61.64^{b}	62.63^{a}	0.566	*
EE	28.64	29.58^{a}	24.24^{b}	20.09^{d}	20.51^{d}	20.86^{d}	21.97^{c}	0.805	*
Ash	17.75	14.77^{d}	15.97^{c}	17.86^{b}	18.84^{a}	17.50^{b}	15.40^{cd}	0.359	*
GE1	572.11	592.47	565.67	539.43	535.47	544.35	560.38	16.331	NS
GE2	5.7211	5.9247	5.6567	5.3943	5.3547	5.4435	5.6038	0.163	NS

a, b, c, d and e: 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), 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

Data in Table (7) show that increasing dietary N-ZnO levels from 0mg/ kg in the control groups (D1 and D2) to 15mg/ kg (D3 and D4) and 30mg/ kg (D5 and D6) significantly improved energy retention (ER%) and protein productive value (PPV%) in the Nile tilapia ($P< 0.05$). At 28°C, ER% increased to 122.28% and 129.19% in groups D3 and D5, respectively, compared to 100% in the control group D1. Under elevated temperature conditions (34°C), ER% further improved, reaching 139.11 and 154.00% for groups D4 and D6, respectively, relative to D2 (100%).

Likewise, PPV% showed substantial increases with N-ZnO supplementation. At 28°C, fish in groups D3 and D5 achieved PPV% values of 162.60 and 167.26%, respectively, relative to the control group D1. At 34°C, groups D4 and D6 reached PPV% values of 147.44 and 161.40%, respectively, compared to D2 (100%). These results suggest that N-ZnO inclusion enhances energy and protein utilization, particularly under elevated temperature stress.

Table 7. Energy retention (ER) and protein productive value (PPV) % of different experimental groups

Item	Experimental groups						SEM	Sign. $P<0.05$
	Fish fed basal diet and reared in water normal temperature (28°) (control No. 1)	Fish fed basal diet and reared in water temperature (34°) (control No. 2)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in normal water temperature (28°)	Fish fed basal diet and 15 mg N-ZnO particle size and reared in water temperature (34°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water normal temperature (28°)	Fish fed basal diet and 30 mg N-ZnO particle size and reared in water Temperature (34°)		
	G_1	G_2	G_3	G_4	G_5	G_6		
IW	155	154	154	155	156	154	0.478	NS
FW	380^d	358^e	552^c	547^c	592^a	580^b	23.09	*
Calculation theenergy retention								
ECFBW	5.9247^a	5.6567^b	5.3943^{cd}	5.3547^d	5.4435^c	5.6038^b	0.048	*
TEEBF	2251^e	2025^f	2978^c	2929^d	3223^b	3250^a	114.15	*
ECIBF	5.7211							
TESBF	887	881	881	887	892	881	2.703	NS
ERBF	1364^e	1144^f	2097^c	2042^d	2331^b	2369^a	113.93	*
EFI	4.652							
QFI	561.95^c	544.74^d	700.14^b	698.88^b	736.68^a	732.48^a	19.085	*
TEFI	2614^c	2534^d	3257^b	3251^b	3427^a	3407^a	88.78	*
ER%	52.18^e	45.15^f	64.38^c	62.81^d	68.02^b	69.53^a	2.132	*
Calculation the protein productive value (PPV) %								
CPFBC%	55.65^e	59.79^d	62.05^{ab}	60.65^c	61.64^b	62.63^a	0.566	*
PR_1	211.47^d	214.05^d	342.52^b	331.76^c	364.91^a	363.25^a	16.017	*
CPIBFC%	53.61							
PR_2	83.10	82.56	82.56	83.10	83.63	82.56	0.256	NS
PR_3	128.37^d	131.49^d	259.96^b	248.66^c	281.28^a	280.69^a	16.002	*
CPFE%	30.66		30.65		30.64		-	-
TPI	172.30^c	167.02^d	214.59^b	214.21^b	225.72^a	224.43^a	5.838	*
PPV%	74.50^e	78.73^d	121.14^b	116.08^c	124.61^a	127.07^a	5.222	*

a, b, c, d, e and f: 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), Quantity of feed intake, TEFI: Total energy of feed intake (EF)ER%: Energy retention (ER) % CPFBC%: Crude protein % in final body fishPR$_1$: 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.

Economical evaluation of different experimental groups

As shown in Table (8), incorporating nano zinc oxide (N-ZnO) into the feed formulation slightly increased the cost per kilogram of feed from 24.350LE/ kg in the control diets (D1 and D2) to 24.425LE/ kg in diets D3 and D4, and to 24.500LE/ kg in diets D5 and D6. Despite this modest rise in feed cost, considerable improvements in net returns were observed. Net profits increased by 6.78, 29.70, 28.89, 32.60, and 31.40% in groups D2, D3, D4, D5, and D6, respectively. These findings demonstrate that dietary N-ZnO supplementation enhances economic efficiency and provides a favorable return on investment despite the slight increase in feed cost.

Table 8. Economical evaluation of different experimental groups

	Experimental groups					
	Fish fed basal diet and reared in water normal temperature (28°) (control No. 1)	**Fish fed basal diet and reared in water temperature (34°) (control No. 2)**	**Fish fed basal diet and 15 mg N-ZnO particle size and reared in normal water temperature (28°)**	**Fish fed basal diet and 15 mg N-ZnO particle size and reared in water temperature (34°)**	**Fish fed basal diet and 30 mg N-ZnO particle size and reared in water normal temperature (28°)**	**Fish fed basal diet and 30 mg N-ZnO particle size and reared in water temperature (34°)**
Item	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**	**G_6**
Costing of kg feed (LE)	24.350	24.350	24.425	24.425	24.500	24.500
Relative to control (%)	100	100	100.31	100.31	100.62	100.62
Feed conversion ratio (FCR)	2.50	2.67	1.76	1.78	1.69	1.72
Feeding cost (LE) per (Kg weight gain)	60.88	65.01	42.99	43.48	41.41	42.14
Relative to control (%)	100	106.78	70.61	71.42	68.02	69.22
Net improving in feeding cost (%)	Zero	6.78	29.70	28.89	32.60	31.40

Diet formulation calculated according to the local prices at year 2021 as presented in Table (1).
Feed cost (L.E) FCR×FI. Cost per Kg diet

DISCUSSION

The chemical analysis of the experimental diets revealed crude protein levels ranging from 30.647 to 30.66%, and a consistent gross energy value of 4652 kcal/kg. Metabolizable energy (ME) ranged from 372.31 to 372.36 kcal/kg, with slight variation in the protein-to-energy ratio (82.30–82.34 mg CP/kcal ME). These values align with the nutritional requirements of the Nile tilapia, confirming that the diets were both iso-caloric and iso-nitrogenous.

The present results demonstrated that supplementing the diets with nano zinc oxide (N-ZnO) at concentrations of 15 and 30mg/ kg significantly enhanced growth performance in the Nile tilapia maintained at both 28 and 34°C. Notably, the best growth metrics—including final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR)—were observed in group D5 (30 mg/kg N-ZnO at 28°C). Importantly, all fish groups exhibited 0% mortality, indicating that the treatments were safe and had no adverse effects on survival.

Consistent with our findings, several previous studies have demonstrated the beneficial role of both organic and nano forms of zinc in aquaculture. For instance, **Lin *et al.* (2013)** and **Shahpar and Johari (2019)** reported growth improvements in various fish species following zinc supplementation. **Tawfik *et al.* (2017)** found that adding 60mg/ kg of nano-ZnO to the Nile tilapia diets improved weight gain and SGR. Similarly, **Mahavadiya *et al.* (2023)** reported that dietary zinc nanoparticles at 10 and 20mg/ kg improved growth in tilapia fry, although no further improvement was observed at 30mg/ kg. Survival rates, however, remained unaffected across treatments.

Kishawy *et al.* (2020) also showed that nano-ZnO at 20mg/ kg outperformed other zinc sources in enhancing tilapia growth, improving SGR and protein efficiency ratio (PER), and reducing feed conversion ratio (FCR). These effects were attributed to zinc's central role in metabolic processes. Likewise, **Kumar *et al.* (2018)** demonstrated that diets containing 10 or 20mg/ kg zinc nanoparticles improved growth, survival, immune response, and oxidative stress tolerance in *Pangasianodon hypophthalmus*.

Such evidence supports the notion that nano-zinc formulations offer superior bioavailability and physiological benefits in aquafeed. **Pan *et al.* (2022)** found that varying dietary zinc levels (10– 80mg/ kg) from either zinc sulfate or zinc methionine significantly enhanced weight gain, SGR, PER, and FCR, although the zinc source alone had no effect on survival. **Abdel-Hammed *et al.* (2019)** further demonstrated that nanoparticle forms of iron and zinc promoted better growth outcomes than their bulk equivalents.

Zinc's essential role in fish metabolism is well-established. According to **Chanda *et al.* (2015)**, zinc supports key metabolic pathways including prostaglandin metabolism and nucleoprotein stability. **Chesters (1991)** identified it as a vital cofactor for at least 20 metalloenzymes such as alkaline phosphatase and carbonic anhydrase, underscoring its indispensable role in enzyme-regulated processes.

Recent insights into zinc–gene interactions suggest that mineral form significantly impacts bioactivity. Nano-scale minerals, in particular, have demonstrated enhanced effects on growth and immune function due to improved antioxidant properties and better absorption, allowing for lower inclusion levels with equivalent or superior outcomes **(Rather *et al.*, 2011; Rajendran, 2013)**. **Uzo-God *et al.* (2018a)** also reported enhanced weight gain with nano-ZnO compared to conventional zinc oxide. However, a subsequent study **(Uzo-God *et al.*, 2018b)** showed that fish fed Fe_2O_3 outperformed those fed nFe_2O_3, highlighting the variability of nano-mineral effects depending on element and formulation.

Zinc is not only essential but can also be toxic at excessive levels. **Fahmy *et al.* (2014)** emphasized its importance in maintaining physiological homeostasis, noting that imbalances can lead to harmful outcomes in aquatic species. Nevertheless, many studies have endorsed nano minerals—particularly iron and zinc—as promising feed additives. For example, **Huber (2005)** demonstrated that iron nanoparticles improve bioavailability compared to other forms, and **Srinivasan *et al.* (2016)** linked enhanced digestive enzyme activity in *Macrobrachium rosenbergii* post-larvae to nano-mineral supplementation, resulting in better growth and survival.

Selenium nanoparticles have also shown promise. **Dawit Moges *et al.* (2022)** found that Nile tilapia fed 1mg/ kg selenium nanoparticles (Se-NPs) exhibited enhanced growth, SGR, RGR, and FCR, with survival rates exceeding 95% and significant weight gains compared to the control.

In the present study, feed intake (FI) and crude protein intake (CPI) increased with rising N-ZnO supplementation, peaking in group D5 (736.68 and 225.72g, respectively). Correspondingly, FCR improved significantly ($P< 0.05$) in groups supplemented with 15 or 30mg/ kg N-ZnO. PER also improved, with the highest values observed in fish reared at 28°C and fed 30mg/ kg N-ZnO. These results support the conclusion that N-ZnO enhances nutrient utilization efficiency, especially under optimal rearing temperatures.

These findings align with **Kishawy *et al.* (2020)**, who reported that 40mg/ kg zinc supplementation significantly improved weight gain and condition factor (K-factor) in fish. The nano-zinc form exhibited superior outcomes in final body weight (BW), weight gain (WG), SGR, FCR, PER, and K-factor ($P< 0.001$) compared to both organic and inorganic zinc. Notably, no significant differences in feed intake were found ($P> 0.05$).

Similarly, **Pan *et al.* (2022)** evaluated the effects of zinc sulfate heptahydrate (Zn-S) and zinc methionine (Zn-M) across five inclusion levels. Their results showed improved PER and reduced FCR ($P< 0.05$) in shrimp groups supplemented with Zn-S and Zn-M.

Abdel-Hammed *et al.* (2019) compared three levels of nano-iron (nFe_2O_3) and nano-zinc (Nano ZnO) with their bulk counterparts. The best growth and FCR occurred with a combination of 40mg/ kg of both nano forms. Similarly, **Uzo-God *et al.* (2018a)** reported superior weight gain with Nano ZnO over conventional ZnO.

By contrast, **Uzo-God *et al.* (2018b)** found that fish fed bulk Fe_2O_3 had better FCR than those fed nano Fe_2O_3 ($P< 0.05$). Nanoparticles generally exhibit improved intestinal absorption, bioavailability, and catalytic activity **(Dube *et al.*, 2010)**, explaining the advantages seen with Nano ZnO, supported by **Faize *et al.* (2015)**.

Dawit Moges *et al.* (2022) also found improved FCR with 1mg/ kg Se-NPs. **Abdel-Tawwab *et al.* (2010)** showed that zinc proteinate significantly enhanced FCR, PER, and protein retention (PR) at 40mg/ kg ($P< 0.05$). Similarly, **Meiler and Kumar (2021)** reported peak PR in *Oncorhynchus mykiss* at 123mg/ kg zinc proteinate. In this context, **Luo *et al.* (2011)** showed 20.86mg/ kg zinc improved PER in *Pelteobagrus fulvidraco*.

Analysis of body composition revealed no significant effects ($P< 0.05$) on moisture, dry matter (DM), or gross energy (GE), but significant changes ($P< 0.05$) in organic matter (OM), crude protein (CP), ether extract (EE), and ash. Specifically, OM and EE declined, while CP and ash increased, suggesting altered nutrient deposition.

Mahavadiya *et al.* (2023) observed no significant body composition changes in tilapia fry fed 0– 30mg/ kg zinc nanoparticles. In contrast, **Pan *et al.* (2022)** found that zinc source and level significantly influenced CP, crude lipid (CL), and crude ash (CA). Zn-M showed higher bioavailability and immune benefits.

Conflicting reports also exist: **Cui *et al.* (2010)** found zinc levels reduced CL but not CP in muscle; **Guo *et al.* (2013)** found no significant differences in CP, CL, or CA in *Macrobrachium nipponense*; and **Xu *et al.* (2016)** and **Sun *et al.* (2017)** reported no effects on nutrient composition with Zn-M and nano-Zn. Discrepancies may stem from dietary, species, or environmental differences.

Pan *et al.* (2022) suggested that reductions in protein content with Zn-M or Zn-S may relate to zinc's regulatory role in protein metabolism.

Abdel-Khalek *et al.* (2015) reported that Zn NPs accumulated more in liver, gills, and muscles due to better tissue penetration. Similarly, **Abd-Elhamed *et al.* (2021)** observed higher CP and ash in fish fed nano forms. This observation may possibly be attributed to improved enzymatic activity and bioavailability **(Muralisankar *et al.*, 2016)**.

This study also found that increasing N-ZnO from 0 to 30mg/ kg significantly ($P< 0.05$) enhanced energy retention (ER%) and protein productive value (PPV%) in the Nile tilapia. At 28°C, D3 and D5 groups showed ERs of 122.28 and 129.19%, respectively; at 34°C, ER rose to 139.11 and 154.00% in D4 and D6. PPV showed similar increases, reaching 167.26% in D5 and 161.40% in D6. **Abozaid *et al*. (2024)** similarly observed that supplementing tilapia diets with *Saccharomyces cerevisiae* significantly ($P< 0.05$) reduced ER% but enhanced PPV%. In addition, **Abo-State *et al*. (2021)** reported significant differences ($P< 0.05$) in ER and PPV values among different treatments, although supplementation with mannan oligosaccharides (MOS) and β-glucans showed no significant effects ($P> 0.05$). Additionally, **Abozaid *et al*. (2024)** found that net revenue improved by 6.80, 9.47, and 19.03% when yeast was added at 4, 8, and 12g/ kg diet in groups D2, D3, and D4, respectively, compared to the control.

From an economic standpoint, **Goda *et al*. (2012)** highlighted the increasing cost of feed as a major constraint to aquaculture profitability. They recommended supplementing diets with 1g *S. cerevisiae*/100 g feed as the most cost-effective approach for the Nile tilapia fingerlings. **Azevedo *et al*. (2015)** emphasized that economic efficiency is determined by a combination of technical, productive, and price efficiencies, which are derived from multiplying productive by price efficiency.

In the current study, while the inclusion of N ZnO slightly increased feed formulation costs from 24.350 LE/kg in the control diet (D1 and D2) to 24.425 LE/kg (D3 and D4) and 24.500 LE/kg (D5 and D6), it yielded considerable gains in economic returns. Specifically, net economic benefits improved by 6.78, 29.70, 28.89, 32.60, and 31.40% in groups D2, D3, D4, D5, and D6, respectively.

These results are in agreement with **Kishawy *et al*. (2020)**, who found no significant differences ($P > 0.05$) in total feed cost per unit of weight gain among zinc-treated groups. However, diets containing nano-Zn (20 and 40 mg/kg) or organic Zn (40 mg/kg) achieved the highest economic returns and the lowest cost–benefit ratios ($P < 0.001$) compared to lower-dose organic Zn or inorganic zinc treatments. Similarly, **Hassan *et al*. (2017)** demonstrated that rabbits fed nano-ZnO diets exhibited the highest gross margins and lowest production costs per kilogram of live body weight, relative to groups receiving conventional inorganic zinc. The improved cost benefit ratio is likely due to enhanced weight gain rather than differences in feed intake, which remained statistically unchanged across groups.

CONCLUSION

The inclusion of nano zinc oxide (N-ZnO) at levels of 15 and 30mg/ kg in the Nile tilapia diets significantly enhanced growth performance, feed utilization, energy retention, and protein productive value, with even greater benefits observed under elevated water temperatures. Although the use of N-ZnO led to a marginal increase in feed formulation costs, the associated improvements in biological performance and net economic returns more than compensated for this increase. These findings underscore the high bioavailability and metabolic efficiency of zinc in its nanoparticle form, reinforcing its potential as a cost-effective and performance-enhancing dietary supplement in aquaculture. Further investigations are recommended to assess long-term effects, optimize dosing strategies, and evaluate environmental safety across diverse aquatic species and rearing conditions.

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