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Mitigating Heat Stress in the Nile Tilapia (*Oreochromis niloticus*) Using Dietary Nano Zinc Oxide: Impacts on Growth, Biochemistry, and Growth-Related Genes

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ABSTRACT

This study investigated the combined effects of dietary nano zinc oxide (N-ZnO) supplementation and rearing temperature on growth performance, blood biochemistry, and the expression of growth-related genes in the Nile tilapia (*Oreochromis niloticus*). A total of 180 fish (initial weight: 154.7 ± 0.48 g) were randomly assigned to six groups: a control group reared at 28°C, a heat-stressed control at 34°C, and four treatment groups fed diets containing 15 or 30mg/ kg N-ZnO at either 28°C or 34°C. The feeding trial lasted 75 days, with heat stress applied during the final 15 days. N-ZnO supplementation significantly improved final body weight, weight gain, feed intake, and feed conversion ratio, with the most pronounced effects observed at 30mg/ kg under normal temperature conditions. Although heat stress negatively impacted growth parameters, dietary N-ZnO mitigated these adverse effects. Biochemical analyses revealed that elevated temperature reduced serum AST and ALT levels in control fish, whereas N-ZnO supplementation helped restore these enzyme levels toward normal ranges. In contrast, urea, creatinine, and cholesterol levels were not significantly influenced by either temperature or dietary treatment. Gene expression analysis showed that supplementation with 15mg/ kg N-ZnO at 34°C upregulated *IGF1* and *GH* expression in liver tissue, while 30mg/ kg at the same temperature increased *IGF2* expression. In gill tissue, the highest expression levels of *IGF1* and *IGF2* were observed at 30mg/ kg under 28°C, whereas *GH* expression peaked at 15mg/ kg under 34°C. These findings demonstrate that dietary N-ZnO, particularly at 15– 30mg/ kg, enhances growth performance, supports liver function, and promotes the expression of growth-related genes under thermal stress. This suggests its potential as a nutritional strategy for improving tilapia resilience and productivity in warming aquaculture environments.

INTRODUCTION

Water temperature is one of the most influential environmental factors affecting the physiology, metabolism, and growth of aquatic ectothermic organisms including fish. Each species has a preferred thermal range, beyond which biological performance declines. For the Nile tilapia (*Oreochromis niloticus*), a widely farmed warmwater species, optimal growth typically occurs between 26– 30°C **(Bildik *et al.*, 2019)**. Temperatures below 16°C impair growth, and prolonged exposure below 10°C can be fatal. Remarkably, tilapia can tolerate high temperatures up to 40°C, although such conditions often induce physiological stress **(Li *et al.*, 2023)**.

Temperature fluctuations affect not only metabolic processes but also endocrine regulation, particularly the somatotropic axis involving growth hormone (GH) and insulin-like growth factors (IGF1 and IGF2). These hormones play critical roles in regulating growth and development and are known to respond to both environmental and nutritional stimuli **(Gabillard *et al.*, 2003)**. Previous research has shown that temperature changes can alter the expression of *GH* and *IGF* genes in fish, thereby influencing protein synthesis, growth rate, and thermal tolerance **(Kayhan & Atasayar, 2003)**.

To enhance thermal resilience and growth performance, aquaculture practices have increasingly explored the use of dietary supplements, including trace minerals in nanoparticle form. Nano zinc oxide (N-ZnO) has emerged as a promising additive due to its small particle size, high bioavailability, and strong antioxidant and antimicrobial properties. Compared to conventional zinc sources, N-ZnO has demonstrated superior effects on growth, immune function, and feed efficiency in both livestock and aquatic animals **(Rajendran, 2013; Swain *et al.*, 2016)**.

In fish, dietary N-ZnO supplementation has been associated with enhanced growth performance, immune responses, and increased activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) **(Mohammady *et al.*, 2021)**. In the Nile tilapia, supplementation with 15–30 mg/kg N-ZnO has resulted in increased body weight gain, upregulation of immune-related genes such as *IL-1β* and *IgM*, and elevated *GH* expression **(Tawfik *et al.*, 2017)**. Similar benefits have been observed in other species, including grass carp and common carp **(Faiz *et al.*, 2015; Dekani *et al.*, 2019; Swain *et al.*, 2019)**.

These positive effects are thought to result from improved intestinal absorption, enhanced enzymatic activity, and increased nucleic acid synthesis, which collectively promote cellular proliferation and growth. Additionally, N-ZnO plays a key role in mitigating oxidative stress, particularly under environmental stressors such as elevated temperatures **(Swain *et al.*, 2016; Onuegbu *et al.*, 2018)**.

While the individual effects of N-ZnO supplementation and temperature on fish physiology have been widely studied, research on their combined impact remains limited. Given the increasing thermal stress associated with climate change, it is critical to assess

whether dietary strategies such as N-ZnO supplementation can alleviate temperature-induced growth suppression.

Therefore, the present study aimed to evaluate the effects of dietary N-ZnO supplementation at two concentrations (15 and 30mg/ kg) under two rearing temperatures (28 and 34°C) on growth performance, feed utilization, blood biochemical parameters, and the expression of growth-related genes (*IGF1*, *IGF2*, and *GH*) in the liver and gill tissues of the Nile tilapia. This study provides insights into the interaction between dietary nano-supplementation and environmental temperature, with implications for enhancing aquaculture sustainability under climate-induced thermal stress.

MATERIALS AND METHODS

Experimental design and fish rearing

This study was conducted to evaluate the effects of dietary nano zinc oxide (N-ZnO) supplementation at different levels under varying thermal conditions on the growth performance, blood biochemical responses, and gene expression of the Nile tilapia (*Oreochromis niloticus*). The experiment was carried out at the Fish Experimental Laboratory, Animal Production Department, Biological Agriculture Research Institute, National Research Centre (NRC), Egypt. Nano zinc oxide was synthesized in the Department of Animal Production, NRC, by the author, following the protocols described by **Ismail *et al.* (2019)**, **Samy *et al.* (2019, 2022)**, **Menazea *et al.* (2021)** and **Abd-Elsamee *et al.* (2024)**.

A total of 180 healthy Nile tilapia juveniles (initial average weight: 154.67 ± 0.48 g; length range: 18–22 cm) were sourced from the Abbassa Fish Hatchery, Sharkia Governorate, Egypt. Based on their body size and developmental stage, these fish were classified as juveniles to sub-adults. Upon arrival, fish were acclimatized for two weeks under standard laboratory conditions and fed the control basal diet to eliminate any carryover nutritional effects.

Following acclimation, fish were randomly distributed into six experimental groups, with three replicates per group (10 fish per aquarium; 18 aquaria in total). Each group was housed in a glass aquarium (80 × 40 × 30 cm; 60 L capacity) equipped with continuous aeration and maintained with a 30% daily water exchange.

Experimental treatments

The six experimental groups are outlined in Table (1) and were assigned as follows:

- **G1**: Control diet at 28°C
- **G2**: Control diet at 34°C (thermal stress)
- **G3**: 15 mg/kg N-ZnO at 28°C
- **G4**: 15 mg/kg N-ZnO at 34°C
- **G5**: 30 mg/kg N-ZnO at 28°C
- **G6**: 30 mg/kg N-ZnO at 34°C

All experimental diets were formulated to be iso-caloric and iso-nitrogenous. Fish were fed twice daily at a rate of 3% of body weight for a total of 75 days. The elevated

temperature (34°C) was applied only during the final 15 days of the trial for groups G2, G4, and G6 to simulate thermal stress conditions.

Throughout the experimental period, fish were clinically monitored twice daily for behavioral changes, feeding response, signs of stress, and mortality. No clinical abnormalities or mortality were observed during the study.

Table 1. Composition of the different experimental diets

Item	Experimental diets		
	D_1 (Basal Diet) Fed to G_1 and G_2	**D_2 (15 mg N-ZnO/kg) Fed to G_3 and G_4**	**D_3 (30 mg N-ZnO/kg) Fed to G_5 and G_6**
Composition of tested diets			
Soybean meal (44%)	40.00	40.00	40.00
Protein concentration (56%)	17.00	17.00	17.00
Yellow corn (8%)	28.00	28.00	28.00
Wheat bran (13%)	10.00	10.00	10.00
Vegetable oil	3.00	3.00	3.00
Salt (sodium chloride)	1.00	1.00	1.00
Vitamin and Minerals*	1.00	1.00	1.00
Nano Zinc Oxide particle size	00.00	15 mg/kg (basal diet)	30 mg/kg (basal diet)

** 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.

Growth performance and feed utilization

Growth performance was monitored weekly by recording the individual body weight and total length of all fish. The following parameters were calculated:

Body weight gain (BWG) = Final weight (g) – Initial weight (g)

Specific growth rate (SGR) = ln(Finalweight)–ln(Initialweight)ln(Final weight) – ln(Initial weight)ln(Finalweight)–ln(Initialweight) / Days × 100

Feed conversion ratio (FCR) = Total feed intake (g) / Body weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g) / Crude protein intake (g)

Feed efficiency (FE%) = [Weight gain / Feed intake] × 100

Protein productive value (PPV%) = [(Final body protein – Initial body protein) / Protein intake] × 100

These metrics were used to assess the influence of dietary N-ZnO and temperature on growth and nutrient utilization.

Blood sampling and biochemical analysis

At the end of the trial, six fish per treatment group (two fish per replicate) were randomly selected and anesthetized using clove oil (0.5mL/ L). Blood samples were collected from the caudal vein using sterile 3mL syringes and transferred into plain tubes. The samples were allowed to clot at room temperature and then centrifuged at 3000 rpm

for 15 minutes to separate the serum. The serum was stored at −20°C until further analysis.

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), blood urea, creatinine, and total cholesterol (g/dL) were measured using standardized protocols described by **Wu (2006)**, along with commercial diagnostic kits (Spectrum Diagnostics, Egypt). Biochemical endpoints were analyzed according to the manufacturer's instructions using a UV-Vis spectrophotometer (AGILENT CARY 100/300 Series, USA).

Gene expression analysis

To evaluate the molecular response to dietary N-ZnO supplementation and thermal stress, quantitative gene expression analysis was conducted on liver and gill tissues. At the end of the feeding trial, three fish per replicate (nine fish per treatment group) were randomly selected and euthanized via overdose anesthesia (clove oil, 1mL/ L). Liver and gill tissues were immediately excised, snap-frozen in liquid nitrogen, and stored at −80°C for subsequent RNA extraction.

Total RNA was extracted using TRIzol™ reagent (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's protocol. The concentration and purity of RNA were assessed spectrophotometrically using a NanoDrop 2000 (Thermo Fisher Scientific) by measuring the A260/A280 ratio. RNA integrity was verified through 1% agarose gel electrophoresis.

Complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) with 1µg of total RNA per reaction, following the manufacturer's instructions.

Quantitative real-time PCR (qPCR) was performed using the QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) with SYBR Green Master Mix (Roche). Each 20µL PCR reaction contained 10µL of SYBR Green Master Mix, 1µL each of forward and reverse primers, 2µL of cDNA template, and 6µL of nuclease-free water. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds.

The target genes analyzed included *IGF1*, *IGF2*, and *GH*, with *β-actin* serving as the internal control (housekeeping gene). Relative expression levels were calculated using the 2^-ΔΔCt method **(Livak & Schmittgen, 2001)**. Primer sequences were designed based on GenBank accession numbers, as detailed in Table (2).

Table 2. Primer sequences used for gene expression analysis

Gene	Primer Sequences	Accession no.
GH	F: CTGTCTGTCTGTCTGTCAGTCGT R: AGAGGAGACGCCCAAACAC	M97766.1
IGF-1	F: CCCGAACTTCCTCGACTTGA R: CCTCAGCCAGACAAGACAAAAA	AF033797
IGF-2	F: CCCCTGATCAGCCTTCCTA R: GACAAAGTTGTCCGTGGTGA	EU272150
β-actin	F: ACCCACACAGTGCCCATC R: CAGGTCCAGACGCAGGAT	EF026001.1

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS software (Version 22.0, SPSS Inc., USA). Differences between treatment means were determined using Duncan's multiple range test at a significance level of $P< 0.05$ **(Duncan, 1955)**. Results are expressed as mean ± standard error (SE).

RESULTS

Chemical analysis of different experimental diets

Data presented in Table (3) show that crude protein levels in the experimental diets ranged from 30.647 to 30.66%. All three diets provided a gross energy value of 4652 kcal/kg. Metabolizable energy (ME) values varied slightly, ranging from 372.31 to 372.36 kcal/kg. Additionally, the protein-to-energy ratio ranged between 82.30 and 82.34 mg CP/kcal ME. These values are considered appropriate to meet the nutritional requirements of the Nile tilapia. Furthermore, the diets can be classified as iso-caloric and iso-nitrogenous, ensuring consistent energy and protein content across all treatments.

Table 3. Chemical analysis of different experimental diets

Item	Experimental diets		
	D_1 (Basal Diet) Fed to G_1 and G_2	**D_2 (15 mg N-ZnO/kg) Fed to G_3 and G_4**	**D_3 (30 mg N-ZnO/kg) 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 NRC (2011). 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 performance and feed utilization

Data presented in Table (4) indicate that dietary inclusion of nano zinc oxide (N-ZnO) significantly enhanced growth performance in Nile tilapia. Final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) were all significantly improved in fish fed diets supplemented with 15 or 30mg/ kg N-ZnO at both 28 and 34°C, compared to the respective control groups (G1 and G2) that received no N-ZnO.

Notably, the group receiving 30mg/ kg N-ZnO and reared at 28°C (G5) exhibited the highest values across all measured growth parameters. This group outperformed both control groups and the other treatment groups (G3, G4, and G6), indicating a synergistic effect of optimal temperature and higher N-ZnO supplementation. Importantly, no mortality was recorded in any experimental group during the entire trial period.

In terms of feed utilization, both feed intake (FI) and crude protein intake (CPI) increased with higher levels of dietary N-ZnO. Fish in groups fed 15mg/ kg (G3 and G4) or 30mg/ kg (G5 and G6) N-ZnO diets showed elevated FI and CPI compared to control groups (G1 and G2). The highest values were recorded in group G5, with an FI of 736.68g and CPI of 225.72g.

Feed conversion ratio (FCR) improved significantly ($P< 0.05$) in fish receiving N-ZnO-supplemented diets, indicating more efficient feed utilization compared to unsupplemented controls. Similarly, protein efficiency ratio (PER) increased significantly ($P< 0.05$) with rising N-ZnO levels—from 0mg/ kg in G1 and G2, to 15mg/ kg in G3 and G4, and reaching the highest in G5 and G6 at 30mg/ kg. The most favorable FCR and PER values were recorded in group G5, further reinforcing the benefits of 30mg/ kg N-ZnO at 28°C.

Table 4. Live body weight, feed intake & utilization, specific growth rate and relative growth rate of the Nile tilapia *(O. niloticus)* fed diets contained different concentrations of nanozinc and reared in different water temperature

	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 watertemperature (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**		
Number of fish	**30**	**30**	**30**	**30**	**30**	**30**	-	-
Live body weight, g								
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	*
Durationexperimental				**75 days**				
ADG, g	3.00^{d}	2.72^{e}	5.31^{c}	5.23^{c}	5.81^{a}	5.68^{b}	0.307	*
Feed intake and utilization								
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	*
Specific growth rate and Relative growth								
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	*

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. FI: Feed intake, FCR: Feed conversion ratio, FCP%: Feed crude protein percentages, CPI: Crude proteinintake.PER: Protein efficiency ratio.

Blood sampling and biochemical analysis

At the end of the trial, six fish per treatment group (two fish per replicate) were randomly selected and anesthetized using clove oil (0.5 mL/L). Blood samples were collected from the caudal vein using sterile 3 mL syringes and transferred into plain tubes. The samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 15 minutes to separate the serum. The resulting serum was stored at −20°C until further analysis.

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), blood urea, creatinine, and total cholesterol (g/dL) were measured following standardized protocols described by **Wu (2006)**, using commercial diagnostic kits (Spectrum Diagnostics, Egypt). Biochemical reaction endpoints were determined according to the manufacturer's instructions using a UV-Vis spectrophotometer (AGILENT CARY 100/300 Series, USA).

Table 5. Changes in biochemical parameters of the Nile tilapia *(O. niloticus)* fed diets contained different concentrations of Nanozinc and reared in different water temperature

	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 sizeandreared 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**
Albumin (g/dl)	0.75^{a}± 0.1	0.50^{bc}±0.1	0.65^{ab} ± 0.1	0.55^{b} ± 0.08	0.50^{bc} ±0.09	0.35^{c}± 0.05
Liver function						
AST (IU/L)	141^{a} ± 7.1	96.5^{b} ± 3.5	49^{c} ± 7.1	58^{c} ± 7.1	90.5^{b} ± 12.0	166^{a} ± 17.0
ALT (IU/L)	16.5^{b}± 2.1	11.5^{d} ± 0.7	8.5^{e} ± 0.7	11.5^{d} ± 0.7	14.0^{c} ±1.4	18.5^{a} ±2.1
Kidneys function						
Creatinine (mg/dl)	0.45^{a}± 0.1	0.35^{ab} ± 0.1	0.15^{c} ± 0.05	0.25^{bc} ±0.03	0.15^{c} ± 0.05	0.35^{ab} ± 0.06
Urea (mg/dl)	5.5^{a} ± 0.7	4.0^{ab} ± 1.4	2.5^{b} ± 0.1	3.5^{ab}±0.7	4.0^{ab} ± 1.4	3.5^{ab} ± 0.7
Total Cholesterol (mg/dl)	246.5^{a} ± 12.0	171.5^{b} ± 4.9	128^{d} ± 8.5	149^{c} ±12.7	153^{c} ± 21.2	143^{c} ±14.1

Note: Data are expressed as mean ± standard division.
a, b, c, d and e: Means in the same row having different superscripts differ significantly ($P < 0.05$).
*: Significant at $P< 0.05$.
AST: Aspartate aminotransferase (AST).
ALT: Alanine aminotransferase.

Levels of gene expressions

Liver tissues

The expression levels of *IGF1*, *IGF2*, and *GH* genes in the liver tissue of the Nile tilapia fed basal diets supplemented with either 15mg/ kg or 30mg/ kg N-ZnO under two temperature regimes (28 and 34°C) are presented in Fig. (1). Gene expression was evaluated across six treatment groups.

IGF1 gene expression

In group G3 (15 mg/kg N-ZnO at 28°C), *IGF1* expression was significantly down-regulated compared to the control (G1). Conversely, group G4 (15 mg/kg N-ZnO at 34°C) exhibited a significant up-regulation of *IGF1* expression relative to the heat-stressed control (G2). Similarly, group G5 (30 mg/kg N-ZnO at 28°C) showed a marked down-regulation of *IGF1* expression, while group G6 (30 mg/kg N-ZnO at 34°C) demonstrated a significant up-regulation compared to the control group.

IGF2 gene expression

In group G3, *IGF2* expression was significantly down-regulated relative to the control. Group G4 showed a non-significant reduction in expression compared to the control. Group G5 also resulted in a significant suppression of *IGF2* expression. However, group G6 exhibited a significant up-regulation of *IGF2* compared to the heat-stressed control.

GH gene expression

GH gene expression was significantly reduced in group G3 compared to the control. In contrast, group G4 showed a significant increase in *GH* expression. Both G5 and G6 exhibited reduced *GH* expression levels compared to the respective controls. The down-regulation in G5 was statistically significant, whereas the reduction observed in G6 was not statistically significant.

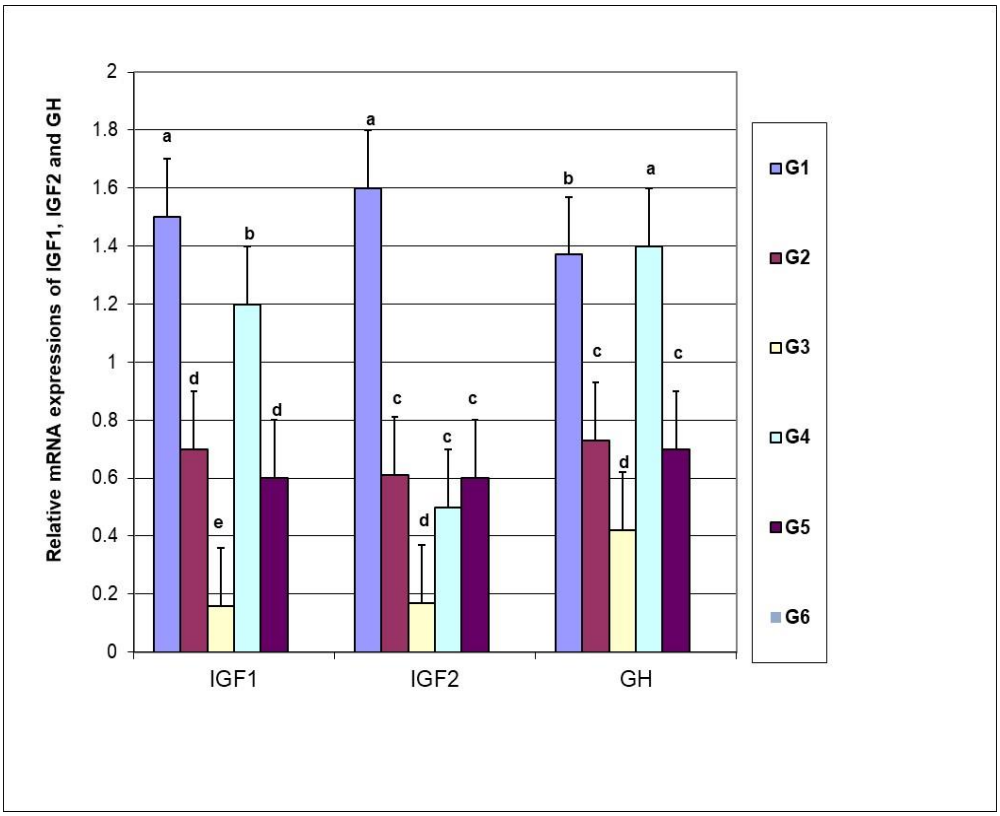

Fig. 1. Relative mRNA expression of IGF1, IGF2, and GH genes in liver tissues of the farmed Nile tilapia fish after feeding diets with different N-ZnO concentrations (15 and 30 mg/kg) at two different incubation temperature degrees (28^{o}C and 34^{o}C). Different letters are significantly different at $P< 0.05$

Gill tissues

The expression levels of *IGF1*, *IGF2*, and *GH* genes in the gill tissues of the Nile tilapia fed a basal diet supplemented with either 15 or 30mg of nano zinc oxide (N-ZnO) under two water temperatures (28 and 34°C) are illustrated in Fig. (2). The results correspond to four treatment groups: G3 (15 mg N-ZnO at 28°C), G4 (15mg N-ZnO at 34°C), G5 (30mg N-ZnO at 28°C), and G6 (30mg N-ZnO at 34°C).

***IGF1* gene expression**

In the gill tissue, *IGF1* expression was significantly up-regulated in groups G3 and G5 (15 and 30mg N-ZnO at 28°C), with $P < 0.05$ and $P < 0.001$, respectively, compared to the control. In contrast, group G4 (15mg N-ZnO at 34°C) showed no statistically significant change in *IGF1* expression. However, group G6 (30mg N-ZnO at 34°C) exhibited a marked down-regulation ($P < 0.001$) relative to the control group.

***IGF2* gene expression**

Relative *IGF2* expression was significantly elevated in groups G3 and G5, with G5 showing the highest expression level ($P < 0.001$ and $P < 0.0001$, respectively). Conversely, fish reared at the higher temperature and supplemented with 15mg (G4) or 30mg (G6) of N-ZnO displayed a significant reduction in *IGF2* expression ($P < 0.05$ and $P < 0.01$, respectively) compared to the control.

***GH* gene expression**

Gill *GH* expression was significantly up-regulated in group G3 ($P < 0.05$) relative to the control. No significant change was observed in group G5. Interestingly, both G4 and G6 (15 and 30mg N-ZnO at 34°C) showed strong up-regulation of *GH* expression, with $P < 0.0001$ and $P < 0.001$, respectively, the highest expression being recorded in group G4.

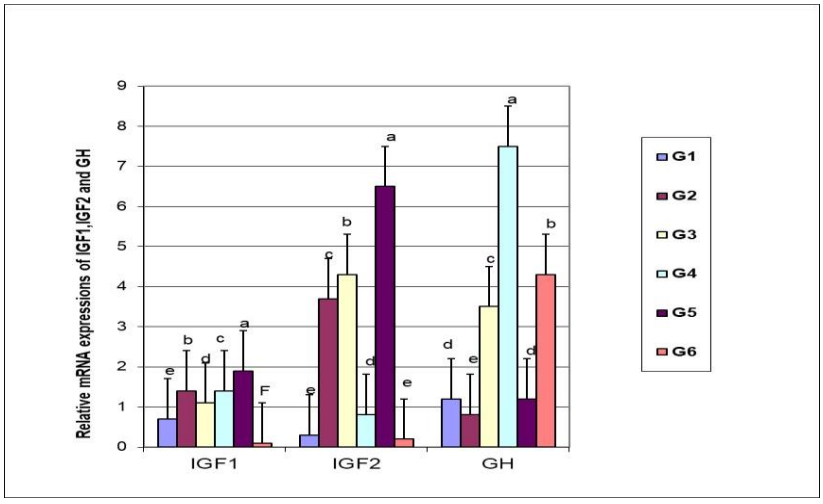

Fig. 2. Relative mRNA expression of IGF1, IGF2, and GH genes in gill tissues of farmed Nile tilapia fish after feeding diets with different N-ZnO concentrations (15 and 30mg/ kg) at two different incubation temperature degrees (28 and 34ºC). Different letters are significantly different at $P < 0.05$

DISCUSSION

The crude protein content of the three experimental diets ranged narrowly from 30.647 to 30.66%. All diets recorded a gross energy value of 4652 kcal/kg, while metabolizable energy (ME) ranged slightly between 372.31 and 372.36 kcal/kg. The protein-to-energy

ratio (mg CP/kcal ME) ranged from 82.30 to 82.34, indicating that all diets were formulated to be isoenergetic and isonitrogenous and were sufficient to meet the nutritional requirements of the Nile tilapia.

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)—were enhanced in fish fed diets supplemented with nano zinc oxide (N-ZnO) at 15 or 30mg/ kg under both temperature conditions (28 and 34°C), compared to the control groups without N-ZnO. Notably, the group receiving 30mg/ kg N-ZnO and reared at 28°C (G5) exhibited the highest values across all growth metrics, outperforming both control groups (G1 and G2) and other supplemented treatments (G3, G4, and G6).

Feed intake (FI) and crude protein intake (CPI) increased progressively with higher dietary N-ZnO levels, with group G5 recording the highest values (736.68g for FI and 225.72g for CPI). Feed conversion ratio (FCR) improved significantly ($P > 0.05$) in groups receiving N-ZnO-supplemented diets compared to controls. Similarly, protein efficiency ratio (PER) increased significantly ($P < 0.05$) with rising N-ZnO levels, with the most favorable FCR and PER observed in group G5.

These findings align with those of **Kishawy *et al.* (2020)**, who emphasized the significance of trace mineral bioavailability, particularly in relation to the mineral's chemical form. They reported that both organic zinc (40mg/ kg) and nano-ZnO (20 and 40mg/ kg) were more effective than inorganic forms in promoting growth performance in *O. niloticus*. The observed improvements are likely associated with enhanced somatic growth through stimulation of DNA/RNA synthesis and cellular proliferation **(Kumar *et al.*, 2018)**.

Numerous studies support the benefits of organic and nano zinc on fish performance. **Shahpar and Johari (2019)** highlighted the growth-promoting potential of both organic and nano zinc sources. **Tawfik *et al.* (2017)** reported that nano-ZnO at 60 mg/kg significantly improved weight gain and SGR in the Nile tilapia. **Mahavadiya *et al.* (2023)** found that diets supplemented with 10–20 mg/kg zinc nanoparticles enhanced growth, while 30 mg/kg showed no additional benefit. **Kishawy *et al.* (2020)** also demonstrated superior growth with 20 mg/kg nano-ZnO over other zinc sources. **Pan *et al.* (2022)** evaluated the effects of zinc sulfate heptahydrate (Zn-S) and zinc methionine (Zn-M) at 10– 80mg/ kg and found both significantly enhanced WGR and SGR. While zinc source alone did not influence survival rate ($P > 0.05$), zinc level significantly affected WGR, SGR, FCR, and PER ($P < 0.05$).

Chesters (1991) and **Chanda *et al.* (2015**) emphasized zinc's involvement in numerous metabolic processes, including its structural role in nucleoproteins and function as a cofactor in over 20 metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase. Recent studies further highlight zinc's role in gene regulation and growth mechanisms.

Uzo-God *et al.* (2018a) reported higher weight gain with nano-ZnO compared to standard zinc oxide, although a later study **(Uzo-God *et al.*, 2018b)** found that iron oxide supplementation had more favorable growth effects than nano iron oxide. **Omer *et al.* (2025)** showed that dietary N-ZnO at 15 and 30mg/ kg significantly improved growth, feed utilization, energy retention, and protein productivity, particularly under elevated temperatures. Similarly, **Dawit Moges *et al.* (2022)** demonstrated that selenium nanoparticles (1 mg/kg) enhanced RGR, SGR, and FCR, along with survivability (>95%) and favorable allometric growth (b = 2.81). Natural additives like cinnamon **(Abozaid *et al.*, 2024a)**, thyme **(Abozaid *et al.*, 2024b)**, and Ashwagandha root powder **(Abozaid *et al.*, 2024c)** also improved growth and feed efficiency.

The significant reduction in AST and ALT levels in fish reared at 34°C compared to ambient controls suggests heat-induced liver function alteration. While higher temperatures may initially increase metabolic activity, prolonged exposure can lead to enzyme denaturation and hepatic stress **(Alkhshali *et al.*, 2019)**. This decline likely reflects hepatocellular stress via oxidative mechanisms **(Li *et al.*, 2023)**. Interestingly, fish fed N-ZnO under heat stress exhibited increased AST and ALT, suggesting that the antioxidant properties of nano-zinc may help counteract oxidative damage, and the enzyme elevation may represent an adaptive hepatic response **(Phornphan *et al.*, 2021)**. Despite transaminase fluctuations, serum albumin levels remained stable across all groups, indicating that hepatic protein synthesis was not significantly impaired **(Teodósio *et al.*, 2022)**. Blood urea and serum creatinine levels were also unaffected by temperature or N-ZnO, suggesting no renal impairment. These are reliable indicators of kidney function, with elevated levels typically indicating renal dysfunction **(Souza *et al.*, 2019)**. Therefore, neither the thermal stress nor N-ZnO supplementation elicited nephrotoxic effects.

Serum cholesterol levels showed a non-significant decrease in all groups, indicating that lipid metabolism remained largely unaffected. Cholesterol is vital for membrane integrity and hormone synthesis, and its stability suggests maintained metabolic homeostasis **(Ramli *et al.*, 2024)**.

Kishawy *et al.* (2020) found that increasing zinc intake, especially from nano and organic sources, significantly elevated serum protein, albumin, globulin, and GH levels, while reducing AST, ALT, and creatinine. Growth hormone increased in a dose-dependent manner ($P < 0.001$). **Zakaria *et al.* (2017)** attributed increased protein levels to enhanced hepatic synthesis and also noted reduced transaminase and creatinine with organic Zn. However, **Das *et al.* (2014)** found no significant effect of organic Zn on AST and ALT in broilers, suggesting species-specific responses.

Pan *et al.* (2022) also reported that Zn-S and Zn-M increased serum TG and CHOL, and enzyme activity (ACP, AKP, SOD), with significant Zn source-level interaction. In contrast, **Dawit Moges *et al.* (2022)** found that Zn-NPs reduced total cholesterol, LDL, and TG, while increasing HDL—indicating lipid-modulatory benefits.

Safawo *et al.* (2018) and **Mahboub *et al.* (2020)** noted that Zn-NPs modulate lipid metabolism through antioxidant mechanisms and regulation of enzymes like lipoprotein lipase and fatty acid synthase. **Ramana *et al.* (2023)** observed improved liver function and reduced serum cholesterol with ZnO-NP supplementation in birds, while **Dawit Moges *et al.* (2022)** warned that excessive Zn-NP intake (40– 50mg/ kg) may cause biochemical stress.

Heavy metal exposure, including zinc, has also been linked to disrupted lipid profiles. **Levesque *et al.* (2002)** found chronic exposure to Zn, Cu, and Cd in *Perca flavescens* caused elevated serum triglycerides—an indicator of liver dysfunction. Similarly, in *Clarias batrachus*, higher Zn-NP doses elevated ALP, AST, ALT, amylase, lipase, and protease, reinforcing the dose-dependent nature of biochemical responses.

In the current study, the expression of growth-related genes (*IGF1*, *IGF2*, *GH*) was evaluated in liver and gill tissues. In the liver, 15mg/ kg N-ZnO at 34°C yielded the highest *IGF1* and *GH* expression, while 30mg/ kg at 34°C maximized *IGF2* expression. In gills, 30mg/ kg N-ZnO at 28°C upregulated *IGF1* and *IGF2*, while *GH* expression peaked at 15mg/ kg under 34°C. This combined temperature and supplementation approach is novel and has not been previously reported.

Prior studies focused on temperature or supplementation independently. **Gabillard *et al.* (2003)** found no temperature effect on *GH* in the rainbow trout embryos but noted increased *IGFR1a*, *IGFR1b*, and *IGF2* expression. **Bildik *et al.* (2019)** observed seasonal variation in *IGF1* and HSPs in *Sparus aurata*. **Huang *et al.* (2021)** linked increased *IGF1* expression and growth in mirror carp to warmer temperatures. Similar outcomes were reported by **Saera-Vila *et al.* (2007)** in *S. aurata* and the hybrid striped bass, and **Li *et al.* (2023)** identified 26–30°C as optimal for feed conversion and weight gain in the Nile tilapia.

Regarding ZnO-NPs, **Kumar *et al.* (2023**) showed that Zn-NPs alleviated arsenic and ammonia toxicity in *Pangasianodon hypophthalmus* by regulating growth and immune gene expression, reducing HSP70/90, and enhancing antioxidant enzyme activity (SOD, CAT, GST, GPx). **Swain *et al.* (2016)**, **Tawfik *et al.* (2017)** and **Mohammady *et al.* (2021)** all confirmed that nano-ZnO enhances growth, gene expression, and antioxidant responses more effectively than conventional zinc sources.

CONCLUSION

Dietary inclusion of nano zinc oxide (N-ZnO) at 15 or 30mg/ kg significantly improved growth performance, feed cost efficiency, and protein utilization in the Nile tilapia. Biochemical analysis showed stable kidney function and unchanged albumin and cholesterol levels, while AST and ALT increased under heat stress, indicating a metabolic response. The optimal combination of N-ZnO levels and rearing temperature enhanced the expression of key growth-related genes in the Nile tilapia. Specifically,

15mg/ kg N-ZnO at 34°C upregulated IGF1 and GH, while 30mg/ kg at 28 or 34 °C increased IGF1 and IGF2 in both liver and gill tissues, highlighting its potential to boost growth and productivity under varying thermal conditions.

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