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# Modulation of Growth, Blood Biochemistry, and Liver and Gill Growth-Related Gene Expression by Dietary Yeast in Heat-Stressed Nile Tilapia

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#### ABSTRACT

This study investigated the effects of dietary dried yeast on growth performance, blood constituents, and gene expression in the Nile tilapia fries reared under different water temperatures. A total of 180 fries (initial average weight  $146.17 \pm 0.845$  g per aquarium) were distributed into 18 aquaria (10 fries each) and assigned to six treatments: G1, G2, and G3 consisted of a basal diet at 28, 31, and 34°C, respectively, while G4, G5, and G6 received the basal diet supplemented with 1.2% dried yeast for 60 days, followed by exposure to 28, 31, and 34°C, respectively, for 15 days to simulate thermal stress. Growth parameters including final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), relative growth rate (RGR), feed intake (FI), crude protein intake (CPI), and protein efficiency ratio (PER) were significantly improved, while feed conversion ratio (FCR) was reduced in yeast-fed groups (G4-G6). Serum biochemical analysis revealed reduced AST and cholesterol levels with yeast supplementation under elevated temperature, while ALT and albumin showed significant variations. Gene expression analysis showed that 34°C with yeast supplementation was the most favorable condition for upregulating IGF1, IGF2, and GH in liver tissues and GH in gills, whereas 28°C with yeast promoted IGF1 and IGF2 in gills. Additionally, 31–34°C with yeast improved liver and kidney functions. Overall, yeast supplementation, particularly at 34°C, enhanced growth, blood biochemistry, and growth-related gene expression, supporting its application in aquaculture under thermal stress.

#### INTRODUCTION

Fish are poikilothermic organisms whose metabolism, growth, and immunity are closely regulated by the thermal conditions of their aquatic environment (Khallaf et al.,







**2021**; **Abozaid** *et al.*, **2025**). Temperature is therefore one of the most critical environmental factors in aquaculture, directly influencing feeding activity, feed conversion, reproduction, and disease resistance.

The Nile tilapia (*Oreochromis niloticus*), a member of the Cichlidae family, is among the most important warm-water fish cultured worldwide (FAO, 2003; Azaza et al., 2008). This species originates from tropical and subtropical freshwater habitats and demonstrates tolerance to a wide thermal range, surviving up to 40°C (Azaza, 2004). However, growth is severely reduced below 16°C, and mortality occurs below 10°C (Chervinski, 1982). The optimal rearing temperature range has been reported between 24–32°C (Chilton & Beamish, 1982), with superior growth and feed utilization at 26–30°C compared to either cooler or warmer conditions (Azaza et al., 2008; Pandit & Nakamura, 2010). More recently, Khallaf et al. (2021) confirmed that maximum performance is achieved at 30–34°C, while temperatures above this threshold impair growth. Identifying effective strategies to support tilapia production under varying temperature conditions is therefore essential for aquaculture sustainability.

In addition to environmental management, feed quality remains a cornerstone of aquaculture performance. Feeds account for the largest share of production costs, and their formulation strongly affects growth efficiency, survival, and profitability (Abozaid et al., 2025b; El-Nadi et al., 2025a, b). Functional feed additives, particularly probiotics, have attracted increasing attention for their ability to enhance growth, immunity, and disease resistance while reducing reliance on antibiotics (Nguyen et al., 2020; Hossain et al., 2020a, b). Probiotics such as Saccharomyces cerevisiae (Sc) are widely recognized as effective and safe dietary supplements in aquaculture (Abu-Elala et al., 2013; El-Wardany et al., 2016; Yang et al., 2020).

Saccharomyces cerevisiae is a yeast historically used in food and pharmaceutical industries, valued for its high content in biologically active compounds such as selenium, zinc, glutathione, β-glucans, and mannan-oligosaccharides (**Agbor** et al., 2007; **Lai** et al., 2008; **Banu** et al., 2020; **Fath** El-Bab et al., 2022). These compounds provide multiple functional benefits. β-glucans have been reported to enhance growth and immune responses in poultry and fish (**Ezzat** et al., 2024). On the other hand, mannan-oligosaccharides exert antimicrobial, antiviral, and immunomodulatory effects (**Patel** & Goyal, 2011; **Meyer** et al., 2015). In aquaculture, S. cerevisiae supplementation has been shown to improve growth performance, feed utilization, and disease resistance in the Nile tilapia and other cultured species (**Abdel-Tawwab** et al., 2008; **Eshak** et al., 2010; **Ozório** et al., 2012; **Iwashita** et al., 2015; **Abass** et al., 2018; **Sutthi** & **Thaimungpbol**, 2020; **Islam** et al., 2021; **Abozaid** et al., 2024; **Ghaly** et al., 2024).

At the molecular level, recent studies have demonstrated that *S. cerevisiae* supplementation influences the expression of growth-related and immune genes (**Cuesta et al., 2003**). For example, significant upregulation of IGF1, IGF2, and GH has been observed in tilapia, while sea bream showed enhanced IL-1β expression following yeast

supplementation (Fath El-Bab et al., 2022; Ghaly et al., 2024). These findings suggest that the growth-promoting effects of yeast are mediated not only through improved feed efficiency but also via modulation of key genetic pathways controlling growth and immunity.

Although both temperature and dietary yeast supplementation play vital roles in tilapia aquaculture, their interactive effects remain poorly understood. Temperature determines the metabolic potential of fish, while yeast provides nutritional and immunological support that may mitigate the negative impacts of thermal stress. A better understanding of this interaction could provide aquaculture with practical tools to optimize growth performance and resilience under fluctuating environmental conditions. The present study was therefore conducted to evaluate the combined effects of rearing temperature and dietary dried yeast (*Saccharomyces cerevisiae*) supplementation on the Nile tilapia fries. Specifically, it assessed growth performance, feed utilization, blood biochemical markers, and the expression of growth-related genes (IGF1, IGF2, and GH), thereby providing new insights into strategies for improving fish productivity and health under thermal stress.

## **MATERIALS AND METHODS**

### **Experimental site and collaboration**

The experiment was conducted at the Fish Experimental Laboratory, Animal Production Department, Biological Agriculture Research Institute, National Research Centre (NRC), Dokki, Cairo, Egypt in collaboration with the Cell Biology Department and Hydrobiology Department, Veterinary Research Institute, NRC.

#### **Experimental fish and acclimation**

A total of 180 healthy Nile tilapia (*Oreochromis niloticus*) fries were obtained from Abbassa Fish Hatchery, Sharkia Governorate, Egypt. Fish were transported to the laboratory and acclimated for two weeks in glass aquaria ( $80 \times 40 \times 30$ cm; water volume: 60L) containing dechlorinated tap water. During acclimation, fish were maintained on a control diet to stabilize their physiological status. Following acclimation, fish (average initial weight:  $146.17 \pm 0.845$  g per aquarium) were randomly distributed into 18 aquaria at a stocking density of 10 fries per tank.

## **Experimental design and diets**

The experiment lasted for 75 days, from early April to mid-June 2024. Fish were divided into six groups (three replicates per group), as follows:

- **G1:** Control, basal diet without yeast, reared at 28°C
- **G2:** Control, basal diet without yeast, reared at 31°C
- **G3:** Control, basal diet without yeast, reared at 34°C
- **G4:** Basal diet + 1.2% dried yeast (12 g/kg), reared at 28°C
- **G5:** Basal diet + 1.2% dried yeast (12 g/kg), reared at 31°C

**G6:** Basal diet + 1.2% dried yeast (12 g/kg), reared at 34°C

All fish were initially acclimated at 28°C for 60 days. Following this acclimation period, fish in the treatment groups were gradually exposed to elevated temperatures of either 31 or 34°C for 15 days, while the control group remained at 28°C for the entire 75-day experimental period. Thus, the "stress" period refers specifically to the final 15 days of exposure to 31 or 34°C after the acclimation at 28°C.

Diets were formulated to be iso-nitrogenous and iso-caloric, with dried yeast incorporated at 1.2% of the diet. Yeast supplementation at 12g/ kg was administered consistently throughout the entire 75-day experimental period. This included both the 60-day acclimation phase and the subsequent 15-day thermal exposure phase. Therefore, the treatment groups (G4–G6) received continuous yeast supplementation without withdrawal at any stage.

The proximate composition of the experimental diets is shown in Table (1). All aquaria were continuously aerated with air pumps, and 30% of the water volume was exchanged daily with fresh dechlorinated water to maintain water quality. Dissolved oxygen, pH, and ammonia were monitored weekly and maintained within the optimal range for the Nile tilapia culture.

Table 1.	Composition	of the	different	experimental diets

	Experimental diets								
Item	$\mathbf{D}_1$	$\mathbf{D}_2$							
	Fed to G <sub>1</sub> , G <sub>2</sub> and G <sub>3</sub>	Fed to G <sub>4</sub> , G <sub>5</sub> and G <sub>6</sub>							
	Basal diet	Tested diet							
Composition of tested diets									
<b>Protein concentration (56%)</b>	17.00	17.00							
Soybean meal (44%)	40.00	40.00							
Ground yellow corn (8%)	28.00	28.00							
Wheat bran (13%)	10.00	8.80							
Vegetable oil	3.00	3.00							
Vitamin and Minerals	2.00	2.00							
mixture*	00.00	1.20							
<b>Dried yeast</b>									

\*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).

#### 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),

The composition of the different experimental diets is presented in Table (1).

## **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)]

### **Blood sampling**

Blood samples were collected from the caudal vein of 18 fish using a 3mL syringe after anesthetization with clove oil (0.5mL/L). Samples were placed in clean, dry centrifuge tubes at room temperature until clotting occurred, then centrifuged at 3000rpm for 15min. The separated serum was collected and stored at  $-20^{\circ}\text{C}$  until biochemical analyses.

## **Analytical procedures**

The proximate composition of experimental diets and fish body composition was analyzed according to the methods of the AOAC (2016).

## Biochemical analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), blood urea, creatinine and total cholesterol (gm/dl) were assessed following established protocols (**Wu** *et al.*, **2006**) with commercially available kits (Spectrum Diagnostics, Egypt). The completion of biochemical reactions was determined according to kit instructions using a spectrophotometer (AGILENT CARY 100/300 Series UV-Vis, United States).

### Gene expression analysis

Total RNA was extracted from liver and gill tissues using TRIzol Reagent (Invitrogen, Thermo Fisher Scientific). RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) was synthesized from total RNA using the High-Capacity cDNA Synthesis Kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's protocol. Quantitative real-time PCR (qPCR) was conducted with the Quant Studio<sup>TM</sup> 5 Real-Time PCR System (Applied Biosystems). Each  $20\mu$ L reaction mixture contained  $10\mu$ L SYBR Green I Master Mix (Roche Diagnostics GmbH),  $1\mu$ L of each forward and reverse primer,  $2\mu$ L cDNA template, and  $6\mu$ L nuclease-free water. The thermal cycling program consisted of an initial denaturation at 95°C for 10min, followed by 40 cycles of 95°C for 15s, 60°C for 30s (annealing), and 72°C for 30s (extension).  $\beta$ -Actin was used as the housekeeping gene for normalization, and relative gene expression levels were calculated using the  $2^{-\Delta\Delta Ct}$  method, as described by **Livak and Schmittgen** (2001). The primer sequences used for gene expression analysis are provided in Table (2).

Gene	Primer Sequences	Accession no.	Reference
GH	F: CTGTCTGTCTGTCAGTCGT R: AGAGGAGACGCCCAAACAC	M97766.1	Rentier-Delrue <i>et al.</i> , 1989.
IGF-1	F: CCCGAACTTCCTCGACTTGA R: CCTCAGCCAGACAAGACAAAAA	AF033797	Wang et al., 2019.
IGF-2	F: CCCCTGATCAGCCTTCCTA R: GACAAAGTTGTCCGTGGTGA	EU272150	Wang et al., 2019.
β-actin	F: ACCCACACAGTGCCCATC R: CAGGTCCAGACGCAGGAT	EF026001.1	Monteiro et al., 2009.

**Table 2.** Primer sequences used for gene expression analysis

#### Calculated data

## **Energy calculations**

The gross energy (GE, kcal/kg DM) of the experimental diets and fish body composition was estimated according to **Blaxter** (1968) and **MacRae and Lobley** (2003), using the following caloric conversion factors: 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 following the recommendations of the **NRC** (2011), applying conversion factors of 4.50, 8.15, and 3.49 kcal/g for protein, fat, and carbohydrate, respectively. The protein–energy ratio (mg CP/kcal ME) was also determined according to the **NRC** (2011).

## Statistical analysis

All collected data were analyzed using one-way analysis of variance (ANOVA) in SPSS version 26.0 (IBM, 2020). Significant differences among treatment means were identified using Duncan's Multiple Range Test (**Duncan, 1955**). Results were considered statistically significant at P < 0.05.

#### RESULTS

## Chemical analysis of different experimental diets

As presented in Table (3), the crude protein content of the two tested diets was 30.15 and 30.80%, respectively. The corresponding gross energy values were 4559 and 4543 kcal/kg DM, while the metabolizable energy (ME) values were 353.94 and 351.37 kcal/kg DM. Moreover, the protein–energy ratio was calculated at 85.18 and 87.66mg CP/kcal ME for the respective diets. These values are considered adequate to meet the nutritional requirements of the Nile tilapia and indicate that the experimental rations were iso-caloric and iso-nitrogenous, thereby ensuring comparability across treatments.

**Table 3.** Chemical analysis of different experimental diets

	Experimental diets				
Item	$\mathbf{D}_1$	$D_2$			
	Fed to G <sub>1</sub> , G <sub>2</sub> and G <sub>3</sub>	Fed to G <sub>4</sub> , G <sub>5</sub> and G <sub>6</sub>			
Moisture	8.15	9.48			
Dry matter (DM)	91.85	90.52			
Chemical	analysis on DM basis				
Organic matter (OM)	93.66	93.14			
Crude protein (CP)	30.15	30.80			
Crude fiber (CF)	6.55	6.85			
Ether extract (EE)	4.18	4.10			
Nitrogen free extract (NFE)	52.78	51.39			
Ash	6.34	6.86			
Gross energy kcal/ kg DM	4559	4543			
Gross energy cal/ g DM	4.559	4.543			
Metabolizable energy kcal/ kg DM	353.94	351.37			
Protein energy ratio (mg CP/ Kcal ME)	85.18	87.66			

Gross energy (kcal/kg DM) was calculated according to (Blaxter1968; 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**

As shown in Table (4), the inclusion of dried yeast at a level of 12g/kg 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) in fish reared at all tested water temperatures (28, 31, and 34°C; groups G4, G5, and G6) compared to their respective control groups (G1, G2, and G3). Notably, fish fed the yeast-supplemented diet and reared at 34°C (G6) exhibited the highest growth performance values across all parameters (FW, TBWG, ADG, SGR, and RGR) relative to the other groups (G1–G5). Regarding survival, G5 recorded a mortality rate of 6.67%, whereas all other groups (G1, G2, G3, G4, and G6) maintained 100% survival. In terms of feed utilization, fish fed diets containing dried yeast (G4, G5, and G6) also showed higher values of feed intake (FI) and crude protein intake (CPI) compared to their nonsupplemented counterparts (G1, G2, and G3) reared under the same water temperatures. The highest values of feed intake (FI) and crude protein intake (CPI) were observed in fish of group G6, recording 656.05 and 206.06g, respectively. Moreover, the feed conversion ratio (FCR) was significantly (P < 0.05) improved in groups G4, G5, and G6 (yeast-supplemented diets at 28, 31, and 34°C) compared to their corresponding nonsupplemented groups (G1, G2, and G3). Similarly, the protein efficiency ratio (PER) was

significantly (P< 0.05) higher in fish from groups G4, G5, and G6 than in fish from groups G1, G2, and G3 reared at the same water temperatures.

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

		ps						
	Fish fed basal diet and			Fish fed o	Fish fed diet contained 12 g dried			
Item	reared in water			y	yeast/kg diet and			Sign •
	temperature at			reared i	reared in water temperature at			
	(28°C)	(31°C)	(34°C)	(28°C)	(31°C)	(34°C)	M	<i>P</i> <0.
	$G_1$	$G_2$	$G_{13}$	$G_4$	$G_5$	$G_6$		05
Number of fish	30	30	30	30	30	30	-	-
			Live bo	dy weight, g				
IW, g					145	146	0.8	NS
	145	152	144	145			45	
FW, g					$475^{\rm b}$	489 <sup>a</sup>	13.	*
	$367^{d}$	$360^{\rm e}$	363 <sup>de</sup>	469°			96	
TBWG, g		1		1	$330^{b}$	343ª	14.	*
	222°	208 <sup>d</sup>	219 <sup>c</sup>	324 <sup>b</sup>			19	
Duration				77.1				
experimental				75 da	,	4.550	0.1	*
ADG, g	2.066	0.77d	2.026	4.22h	$4.40^{b}$	4.57 <sup>a</sup>	0.1	*
	2.96 <sup>c</sup>	2.77 <sup>d</sup>	2.92°	4.32 <sup>b</sup>			89	
TOT	551 46	557.24	Feea intak	e and utilizati		(5( 05)	11	*
FI, g	551.46	557.34	546.42 <sup>d</sup>	627 1 4h	642.18 <sup>b</sup>	656.05 <sup>a</sup>	11.	4
FCR	cu	·	546.42°	637.14 <sup>b</sup>	1.946a	1.913a	48 0.0	*
rck	2.484 <sup>b</sup>	2.680°	2.495 <sup>b</sup>	1.966ª	1.940"	1.915	76	
FCP%	2.404	2.000	2.493	1.900	30.80		30.	
FCF 70	30.15	30.15	30.15	30.80	30.80		30. 80	-
CPI, g	166.27	168.04	30.13	30.80	197.79 <sup>b</sup>	202.06a	3.9	*
C1 1, g	cd	c c	164.75 <sup>d</sup>	196.24 <sup>b</sup>	171.17	202.00	63	
PER			104.75	170.24	1.668a <sup>b</sup>	1.698 <sup>a</sup>	0.0	*
	1.335 <sup>c</sup>	1.238 <sup>d</sup>	1.329°	1.651 <sup>b</sup>	1.0000	1.070	46	
	1.000			ite and Relativ	ve growth			
SGR		ar	, 8		0.92a <sup>b</sup>	0.94ª	0.0	*
~	$0.72^{\circ}$	$0.68^{d}$	$0.72^{\circ}$	$0.91^{b}$	017 <del>- 11</del>		27	
RGR					$2.25^{b}$	2.35 <sup>a</sup>	0.1	*
-	$1.50^{c}$	1.35 <sup>d</sup>	1.50 <sup>c</sup>	$2.20^{b}$			01	
SR %	100	100	100	100	93.33	100	-	-
Dead number	Zero	Zero	Zero	Zero	2	Zero	-	-
Mortality rate							-	-
%	Zero	Zero	Zero	Zero	6.67	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. FI: Feed intake. FCR: Feed conversion ratio. FCP%: Feed crude protein percentages. CPI: Crude protein intake. PER: Protein efficiency ratio.

## Biochemical parameters of the different experimental groups

As shown in Table (5), the present study evaluated the effects of dietary yeast supplementation on the biochemical parameters of *O. niloticus* reared at different water temperatures. The results revealed significant differences in AST activity, which showed a marked decrease with increasing temperature. Yeast inclusion significantly reduced

AST levels in fish reared at room temperature and  $34^{\circ}$ C (G6), while only a slight reduction was observed at  $31^{\circ}$ C (G5) compared to the control at room temperature. In contrast, ALT activity showed no significant differences among most groups, except for the yeast-fed group at  $31^{\circ}$ C (G5), which exhibited a non-significant increase. Serum albumin concentrations displayed a non-significant decrease across most treatments compared to the control at room temperature, whereas a significant increase (P< 0.05) was recorded in the control group at  $31^{\circ}$ C (G2). Regarding kidney function indicators, serum creatinine levels did not differ significantly among treatments, except for a significant decrease in the yeast-fed group at  $31^{\circ}$ C (G5) compared to the control. Similarly, blood urea concentrations showed no significant variation among groups. For metabolic function, serum cholesterol levels significantly (P< 0.05) decreased with rising water temperature in both control and yeast-fed groups relative to the control at room temperature.

**Table 5.** Changes in biochemical parameters of *O. niloticus* fed diets with yeast and exposure to different temperature during the experimental period

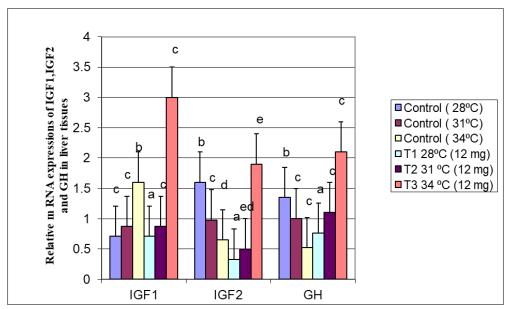
	Experimental groups							
Item	Fish fed basal diet and reared in water temperature at			Fish fed diet contained 12 g dried yeast/kg diet and reared in water temperature at			-	Sign
	(28°C)	(31°C)	(34°C)	(28°C)	(31°C)	(34°C)	SE M	P<0. 05
	$G_1$	$G_2$	<b>G</b> <sub>3</sub>	<b>G</b> 4	G <sub>5</sub>	G <sub>6</sub>		
AST (IU/L)					128.50 <sup>b</sup>	82.50e	6.0	*
	$141.00^{a}$	104.50 <sup>c</sup>	$96.50^{d}$	$68.00^{f}$			94	
ALT (IU/L)					17.50 <sup>a</sup>	$12.00^{bc}$	0.6	*
	$16.50^{a}$	$10.50^{\circ}$	$11.50^{bc}$	$13.00^{b}$			61	
Albumin (mg/dl)					$0.65^{bc}$	$0.55^{\rm cd}$	0.0	*
	$0.75^{b}$	$1.05^{a}$	$0.50^{d}$	$0.45^{d}$			51	
Creatinine					$0.35^{c}$	$0.15^{d}$	0.0	*
(mg/dl)	$0.45^{b}$	$0.55^{a}$	$0.35^{c}$	$0.30^{c}$			31	
Urea (mg/dl)					$5.50^{a}$	$4.50^{\rm b}$	0.2	*
	$5.50^{a}$	$4.50^{\rm b}$	$4.00b^{c}$	$3.50^{\circ}$			04	
Total cholesterol					169.5°	$162.0^{d}$	8.3	*
(mg/dl)	246.5 <sup>a</sup>	$204.0^{b}$	171.5°	139.5 <sup>e</sup>			6	

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

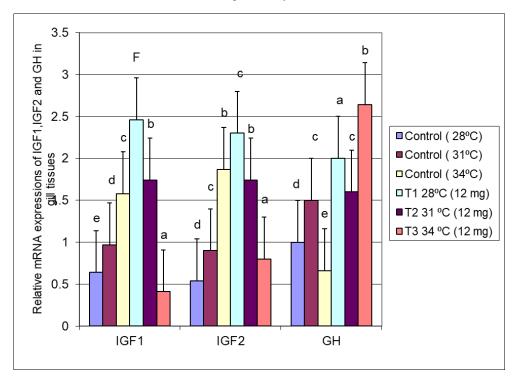
SEM: Standard error of the mean, NS: Not significant, \*: Significant at (P < 0.05), AST: Aspartate aminotransferase

## Levels of gene expressions

The results of IGF-1, IGF-2, and GH gene expression in the liver and gill tissues of *O. niloticus* fed the basal diet with or without yeast (*Saccharomyces cerevisiae*) supplementation under three temperature conditions (28, 31, and 34°C) are summarized in Figs. (1, 2).



**Fig. 1.** Relative mRNA expression of IGF1, IGF2, and GH genes in liver tissues of the farmed Nile tilapia fish after feeding diets contained 12g dried yeast/kg diet at three different incubation temperature degrees (28, 31 and 34°C). Different letters are significantly different at P < 0.05.



**Fig. 2.** Relative mRNA expression of IGF1, IGF2, and GH genes in gill tissues of the farmed Nile tilapia fish after feeding diets contained 12g dried yeast/kg diet at three different incubation temperature degrees (28, 31 and  $34^{\circ}$ C). Different letters are significantly different at P < 0.05.

#### The results of liver tissues

## a. Expression level of IGF-1 gene

The results demonstrated that gene expression in fish exposed to 34°C was significantly upregulated compared to those maintained at 28 and 31°C. Specifically, the expression level of IGF-1 was significantly higher (P< 0.05) in fish reared at 34°C and fed a diet supplemented with 12g/ kg *S. cerevisiae* (G6) compared to G4 and G5. In contrast, the expression levels of IGF-1 in G4 and G5 were similar to those observed in their respective controls. Overall, the conditions of 34°C (control G3) and 34°C with yeast supplementation (G6) were the most favorable for enhancing IGF-1 gene expression, with G6 showing the greatest improvement.

## b. Expression level of IGF -2 gene

The results revealed that the exposure to  $28^{\circ}$ C (control 1) caused significant elevation of gene expression as compared to other controls (31 or  $34^{\circ}$ C). However, the expression level was significantly up regulated (P< 0.05) in G4 with respect to those recorded in other treatments (G4 or G5).

#### C. Expression level of GH gene

Moreover, the expression level of GH gene was significantly up regulated (P< 0.05) at 28°C compared to other temperature degrees (31 or 34°C). However, the expression level was significantly increased in G6 comparing with those observed in the other two treatments (G4 or G5).

## The results of gill tissues

## a. Expression level of IGF-1 gene

The results noted that the exposure to  $34^{\circ}$ C led to high significant (P< 0.05) gene expression as compared to the exposure to 28 or  $31^{\circ}$ C. However, the expression level in the treatment 1 (G4) was revealed to be significantly up regulated in comparison with G5 or G6. The G6 had the lowest gene expression.

#### b. Expression level of IGF-2 gene

Also, the expression level of IGF-2 gene was significantly higher (P< 0.05 or P< 0.01) due to the exposure to temperature degree (34°C) compared to the other two degrees, 28 or 31°C. Whereas the treatment 1 (G4) was recorded with a significant elevation of gene expression comparing with other two treatments G5 or G6. The G6 had the lowest gene expression.

#### C. Expression level of GH gene

The present findings showed that the exposure to 31°C resulted in significant up regulation with respect to exposure to 28 or 34°C. The gene expression level was the lowest at 34°C. Whereas, the treatment 3 (G6) demonstrated the best one, where the gene expression was significantly increased compared to those detected in G4 or G5. G5 had the lowest gene expression level.

#### **DISCUSSION**

The present study investigated the effects of dietary supplementation with Saccharomyces cerevisiae (Sc) at 12g/kg diet under different thermal regimes (28, 31, and 34°C) on growth performance, feed utilization, biochemical parameters, and the expression of growth-related genes (IGF-1, IGF-2, and GH) in the Nile tilapia (Oreochromis niloticus). The chemical analysis of the tested diets revealed that crude protein, energy values, and protein-to-energy ratios were within the optimal range required to support tilapia growth. Importantly, both basal and yeast-supplemented diets were iso-nitrogenous and iso-caloric, which eliminates nutritional variability and emphasizes that observed responses were primarily driven by yeast inclusion and thermal effects rather than dietary imbalance.

Growth performance results clearly demonstrated that the inclusion of S. cerevisiae significantly enhanced final body weight, weight gain, average daily gain, specific growth rate, and relative growth rate across all three water temperatures. The highest values were obtained in fish fed yeast diets at 34°C (G6 group), suggesting a strong synergistic effect of yeast supplementation and elevated temperature (Abozaid et al., 2025b). In contrast, fish reared at the same temperatures without yeast consistently exhibited lower performance indices, highlighting the protective and growth-promoting role of yeast. Feed intake (FI) and crude protein intake (CPI) were the highest in yeastfed fish, reflecting improved diet palatability and nutrient absorption. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were also significantly improved (P < 0.05) in yeast-fed groups, confirming the positive impact of yeast on nutrient utilization efficiency. These results are consistent with previous studies (Lara-Flores et al., 2003; Diab et al., 2006; Davis & Peterson, 2006; Goda et al., 2012; Sutthi & Thaimuangphol, 2020; Abozaid et al., 2024; Abozaid et al., 2025b), which reported that yeast supplementation improves growth rates and feed efficiency in tilapia and other aquaculture species. The beneficial effects can be attributed to yeast-derived bioactive compounds such as \( \beta \)-glucans, mannan oligosaccharides, nucleotides, and vitamins, which act as prebiotics, enhance gut morphology, stimulate enzymatic activity, and improve nutrient bioavailability.

In addition to growth performance, yeast supplementation significantly influenced physiological and biochemical responses. Serum liver enzymes (AST and ALT) are commonly used as indicators of hepatic health and stress. In the present study, AST activity declined significantly with increasing temperature, particularly in yeast-fed fish at 28 and 34°C, indicating reduced hepatocellular damage and better adaptation to heat stress. ALT activity showed non-significant changes across most groups, except for a slight increase at 31°C in yeast-fed fish, which may reflect transient metabolic adjustments rather than pathological damage. Serum albumin levels remained relatively stable, although a significant increase was observed in the control group at 31°C, possibly

reflecting dehydration or altered protein metabolism under thermal stress. Kidney function markers, including blood urea and serum creatinine, showed no major differences among groups, except for a significant reduction in creatinine in yeast-fed fish at 31°C, suggesting a nephroprotective effect of yeast supplementation (**Ramos** *et al.*, **2017**). Cholesterol levels decreased significantly (P< 0.05) with increasing temperature, both in control and yeast-fed fish, reflecting altered lipid metabolism under thermal stress. Interestingly, yeast-fed fish showed lower cholesterol values, supporting earlier findings (**McGill**, **2016**) that yeast  $\beta$ -glucans regulate lipid metabolism and improve cardiovascular health. These biochemical outcomes collectively demonstrate that yeast supplementation enhances physiological resilience and mitigates some negative impacts of high temperature on fish health.

At the molecular level, the expression of growth-related genes IGF-1, IGF-2, and GH was significantly influenced by both dietary yeast and temperature. Expression levels were the highest at 34°C, particularly in fish fed yeast-supplemented diets, where IGF-1 expression was markedly upregulated compared to all other groups. IGF-2 and GH expression followed similar trends, with yeast-fed fish at 34°C showing superior activation compared to their respective controls. These findings suggest that yeast supplementation stimulates the somatotropic axis, enhancing growth-promoting signals under thermal stress conditions. Similar patterns of temperature- and diet-induced gene expression modulation have been reported in the gilthead seabream (Ortuno et al., 2002; Saera-Vila et al., 2007; Bildik et al., 2019), the rainbow trout (Gabillard et al., 2003), and the mirror carp (Huang et al., 2015). The stimulatory effects of S. cerevisiae may be explained by its high content of antioxidants, glutathione, nucleotides, vitamins, and trace elements, which reduce oxidative stress, improve immune responses, and enhance protein synthesis (Lai et al., 2008; Abd-El-Moneim et al., 2017; Fath El-Bab et al., 2022). Interestingly, gene expression results also indicated tissue-specific responses. In liver tissue, IGF-1 was particularly sensitive to the combined effect of yeast and elevated temperature, whereas in gill tissue, yeast supplementation enhanced IGF-1 and IGF-2 expression at 28°C, while GH expression peaked at 34°C. This tissue-dependent regulation may reflect different functional roles of liver and gills in growth and metabolism under stress (Madkour et al., 2024). The liver acts as the central metabolic hub, producing IGF-1 in response to GH stimulation, while gills play an important role in osmoregulation and oxygen exchange, which become more critical under thermal stress. Thus, the observed differences may indicate coordinated physiological adaptation facilitated by yeast supplementation. The overall findings of this study have important implications for sustainable aquaculture. Climate change is expected to increase global water temperatures, posing challenges for tilapia production (Abozaid et al., 2025a, b; Omer et al., 2025). The ability of S. cerevisiae to enhance growth performance, improve biochemical health, and stimulate growth-related gene expression under elevated temperatures suggests its potential as a functional feed additive for climate-resilient aquaculture. Beyond growth, yeast supplementation also provides immunomodulatory and stress-protective benefits, which are critical for maintaining productivity under suboptimal environmental conditions.

#### **CONCLUSION**

The present study demonstrated that dietary supplementation with *S. cerevisiae* at 12g/ kg diet significantly improves growth performance, feed utilization, biochemical health, and gene expression of the Nile tilapia across a range of water temperatures, with the most pronounced effects at 34°C. These findings not only reinforce the role of yeast as a valuable feed additive but also highlight its importance in mitigating the impacts of thermal stress, thereby supporting the development of sustainable aquaculture practices in the face of climate variability.

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