



The palliative role of *Eruca sativa* leaves dietary supplementation against oxidative stress, immunosuppression, and growth retardation in temperature-stressed *Oreochromis niloticus*



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ABSTRACT

This study was conducted to investigate the effects of dietary dried Rocket Leaves meal (DRLM) supplementation on growth performance, immune response, and antioxidant capacity of *Oreochromis niloticus* reared under different water temperature. For this purpose, five hundred and forty apparently healthy *O. niloticus* were allocated into nine groups fed three DRLM-supplemented diets (0, 1, and 3%) and reared at three water temperature (18, 24, and 32°C) in a 3 × 3 total randomized factorial design. The results revealed that exposure of fish to low (18°C) or high (32°C) temperatures for 30 days evoked significant growth retardation, depleted antioxidant enzymes activities (Catalase; CAT and super oxide dismutase; SOD), lipid peroxidation (malondialdehyde; MDA), immunosuppression, altered cortisol level compared to those reared at 24°C. Moreover, a marked down-regulation of oxidative stress related genes with up-regulation of interleukin 1β gene were apparent. In contrast, DRLM incorporation, particularly at 3%, in heat or cold stressed fish diets significantly enhanced growth, restored IgM and lysozymes levels, and SOD and CAT activities. Also, both the MDA and cortisol levels were significantly depressed. Furthermore, both antioxidant and immune-related genes expression were significantly corrected. Conclusively, 3% DRLM dietary supplementation in tilapia diet could be a promising strategy to alleviate the temperature stress-induced negative impacts on fish health and performance.

1. Introduction

Aquaculture is a rapidly growing industry that greatly shares to sustain nutritional safety to the world population (J.S et al., 2018). Nile tilapia (*Oreochromis niloticus*) is a major worldwide species of tilapia used for aquaculture farming and is expected to represent about 62% of total world production in 2030 (FAO, 2018). Currently, rapid climatic variation has a substantial hazard to the environment and to biological organisms particularly fish, for which water temperature is a serious environmental factor (Huang et al., 2018). Furthermore, in the intensive production system, fish are continuously subjected to various stressors. In particular temperature stress, either hyperthermia or hypothermia may induce disturbances of body physiological homeostasis, which could subsequently deter growth and survival (Baras et al., 2001).

The range of 25–30°C has been reported to be the optimal range for *O. niloticus* fingerlings growth and survival (El-Sherif and El-Feky, 2009). Various physiological and biochemical indicators could alter in fish following temperature stress. For example, *O. niloticus* showed a significant alteration in growth performance, hematological, and biochemical profile following exposure to 32°C (Damasceno et al., 2016). Similarly, grass carp showed substantial reductions in serum superoxide dismutase (SOD) and sharp increments in serum glucose when exposed to high-temperature conditions (Cui et al., 2014). Wang et al. (2016) demonstrated that the levels of SOD and malondialdehyde (MDA) increase considerably in heat stressed rainbow trout. Ndong et al. (2007) reported that the activities of serum lysozyme in Mozambique tilapia were substantially suppressed due to cold stress. Recently, Panase et al. (2018) found that rapid decreases in water temperature may impair *O. niloticus* physiology which may further cause mortalities.

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Lately, an increasing interest has been drawn to the incorporation of natural products in tilapia diets to cope with hyperthermia or hypothermia stress maintaining their normal physiological status and growth. For instance, in a recent study, Elabd et al. (2017) revealed *Astragalus membranaceus* and *Glycyrrhiza glabra* dietary incorporation in Yellow perch diet significantly confer protection against different both heat and cold stress. In this regard, *Eruca sativa* (rocket or roquette) is an annual edible plant belongs to the Brassicaceae family and widely distributed in the Mediterranean area (Westberg et al., 2013). In Egypt, *E. sativa* production has been steadily increased for the strong demand for volatile oils for the pharmaceutical purpose (El-Fadaly et al., 2017). *E. sativa* leaves powder were found to contain 15.96% protein, 1.06% fat, 15.66% fiber, 10.71% Ash, 25.67% moisture and 24.95% carbohydrates and mineral content ($\mu\text{g/g}$) were 1.25, 35.71, 655.66, 319.96, and 19.77 of Zn, Fe, Ca, K, and Cu, respectively (ELSadek, 2014). Also, the leaves of this plant have been found to contain substantial amounts of phytochemicals with strong anti-oxidant activities including glucosinolates, polyphenols, flavonoids, isothiocyanates, and carotenoids (Grami et al., 2018). Glucoraphanin, dimeric 4-mercaptobutyl, glucosinolate, 4-*b*-D-glucopyranosylsulfanyl, and glucoerucin are the major glucosinolates (Pasini et al., 2012). Also, in a recent phytochemicals screening of *E. sativa* leaves, gallic acid, kaempferol, quercetin, cirsiolineol, and acacetin have been identified as major compounds of both the polyphenols and the flavonoids groups (Grami et al., 2018). Additionally, isothiocyanates such as sulforaphane and erucin are also present in *E. sativa* leaves extract (Melchini et al., 2009; Villatoro-Pulido et al., 2013). The leaves and seeds of the rocket have been known by several pharmacological effects including anti-ulcer, hepatoprotective, nephroprotective, antidiabetic, repro-protective effects, and diuretics (Alqasoumi et al., 2009; Ansari, 2014; Nowfel and Al-Okaily, 2017; Sarwar Alam et al., 2007). Also, the *E. sativa* leaves methanolic extract has anticholinesterase (Boĝa et al., 2011), anti-inflammatory (Kim et al., 2014) and antithrombotic (Fuentes et al., 2014) activities.

Little is known about the outcomes of incorporating rocket leaves in the fish diet. Fagbenro (2004) reported that substituting 20% of the soybean meal protein with rocket seed meal protein did not alter weight gain, feed conversion ratio, growth response, protein utilization or composition of catfish carcass. However, Khalil et al. (2015) demonstrated the inclusion of 3% dried rocket leaves or 2% dried rocket seeds in *O. niloticus* diets markedly improved their health, growth, nutrient utilization, and chemical composition of the whole fish body.

No available literature exists on the effect of rocket leaves incorporation in the tilapia diet on growth performance, immunity, and oxidative stress when subjected to heat or cold stress. Therefore, the existing study was planned to monitor the effect of incorporating graded levels (0, 1, and 3% kg^{-1} diet) of dried rocket leaves meal (DRLM) in *O. niloticus* diet on their growth, immune response and oxidative reaction at three temperature degrees (18, 24, and 32 °C) with special focusing on the response of oxidative stress and immunity-related genes.

2. Materials and methods

2.1. Diet preparation and feeding regime

The fresh leaves of the rocket were collected from Zagazig city, Sharkia province, Egypt. The leaves have been identified by a taxonomist in the department of horticulture, Faculty of Agriculture, Zagazig University, Egypt. They were cut into small pieces and dried for 72 h at 40 °C then crushed into powder using an electric mixer. The basal diet was prepared to satisfy the *O. niloticus* nutrient needs according to NRC (2011). Three experimental diets were prepared in which the basal diets fortified with 0.0 (control), 1, and 3% DRLM. The composition and proximate analysis of the experimental diets are displayed in Table 1. The ingredients of each diet were carefully mixed

Table 1
Formulation and proximate composition of the experimental diets.

Ingredients	Dried rocket leaves meal level		
	0%	1%	3%
Fish meal	11	11	11
Corn flour	33	32	31
Soybean meal 44%	29	29	28
Corn gluten meal 60%	12	12	12
Wheat bran	8	8	8
Vegetable oil	4	4	4
Dried rocket leaves meal	0	1	3
Vitamin premix ^a	1.5	1.5	1.5
Mineral premix ^b	1.5	1.5	1.5
Total	100	100	100
Proximate composition (% as fed basis)			
Crude protein (N \times 6.25)	30.79	30.91	30.97
Crude lipids	7.43	7.56	7.72
Crude fiber	5.20	5.32	5.36
Ash	5.22	5.78	5.78
Nitrogen free extract ^c	51.36	50.43	50.17
Gross energy (kcal/100 g) ^d	455.3	453.4	454.1

^a Vitamin premix (per kg of premix): vitamin A, 8000000 IU; vitamin E, 7000 mg; vitamin D₃, 2000000 IU; vitamin K₃, 1500 mg; biotin, 50 mg; folic acid, 700 mg; nicotinic, 20000 mg; pantothenic acid, 7000 mg; vitamin B₁, 700 mg; vitamin B₂, 3500 mg; vitamin B₆, 1000 mg; vitamin B₁₂, 7 mg.

^b Mineral premix (per kg of premix): zinc sulfate, 4.0 g; iron sulfate, 20 g; manganese sulfate, 5.3 g; copper sulfate, 2.7 g; calcium iodine, 0.34 g; sodium selenite, 70 mg; cobalt sulfate, 70 mg, and CaHPO₄·2H₂O up to 1 kg.

^c Calculated by difference (100 – protein% + lipids% + ash% + crude fiber %).

^d Gross energy (GE) was calculated as 5.65, 9.45 and 4.11 kcal/g for protein, lipid and NFE, respectively (NRC, 1993).

and 100 ml of water was added per kg diet. Then, the mixture was blended using a kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by laboratory pellet machine at Fish Research Unit, Faculty of Veterinary Medicine, Zagazig University. The pellets were dried in a drying oven for 24 h at 65 °C and kept refrigerated in plastic bags until use. Tested diets were regularly fed twice daily for 30 days. Feeding rate was 3% of the total weight. The diet was applied by hand in corresponding aquaria.

2.2. Fish and experimental conditions

Five hundred and forty apparently healthy *O. niloticus* (an average initial body weight = 35 ± 0.05 g) were attained from Abbassa fish hatchery, Sharkia, Egypt. The fish were stocked in glass aquaria (80 \times 40 \times 30 cm) filled with 60 L of dechlorinated tap water. These were acclimated to a control diet for 2 weeks before the beginning of the trial. The water parameters were monitored and were within the recommended ranges throughout the experiment (pH = 7.1 ± 0.4 ; ammonia = 0.02 ± 0.001 mg/L, nitrite = 0.016 ± 0.001 mg/L). The experiment has been carried out during the winter season in January. The water temperature was lower than 18 °C throughout the experimental period and adjusted by the heater to 18 °C, 24 °C, and 32 °C in different experimental groups. Water exchange was done twice weekly by pre-warmed water and the aquaria were periodically cleaned. During the exposure period, the clinical signs and behaviors were considered. This protocol was accepted by the Ethics of Animal Use in Research Committee of Zagazig University, and experimental procedures were happened in compatibility with the NIH general guidelines for the Care and Use of Laboratory Animals in scientific investigations.

2.3. Experimental design

Fish were randomly distributed into nine groups with three replicate per group (20 fish replicate⁻¹). Group1 (G1): fish fed on basal

Table 2
Oligonucleotide primer sequences and real-time PCR conditions.

Gene name	Primer sequences	Reaction conditions	NCBI accession no.	Reference	
EF-1 α	F	GCTTCAACGCTCAGGTCATC	94°C-15 s/62°C-30 s/72°C-30 s (40 cycles)	AB075952.1	Gröner et al. (2015)
	R	TGTGGCAGTGTGGCAATC			
CAT	F	TCCTGAATGAGGAGGACGA	94°C-15 s/62°C-30 s/72°C-30 s (40 cycles)	JF801726.1	Abdelazim et al. (2018)
	R	ATCTTAGATGAGGCGGTGATG			
SOD	F	GGTGCCTGGAGCCCTA	94°C-15 s/62°C-30 s/72°C-30 s (40 cycles)	JF801727.1	Abdelazim et al. (2018)
	R	ATGCGAAGTCTTCCAATGTC			
IL1 β	F	TGGTGACTCTCCTGGTCTGA	95°C-15 s/60°C-60 s/72°C-30 s (40 cycles)	XM_005457887.1	Standen et al. (2016)
	R	GCACAACCTTTATCGGCTTCCA			

F indicates forward primer; R indicates reverse primer; CAT, catalase; SOD, superoxide dismutase; IL1 β , interleukin 1beta.

Table 3
Growth performance of Nile tilapia fed diets containing three levels of dried rocket leaves meal (DRLM) and reared at 18, 24, and 32°C for 30 days.

Diets	Initial weight (g)	Final weight (g)	Total length (cm)	Weight gain (g)	Weight gain (%)	Specific growth rate (%)	Condition factor	Survival (%)	
Water temperature °C	DRLM %								
Individual treatment means ^a									
18	0	35.23 ± 0.21	38.18 ± 0.29 ^f	13.93 ± 0.11	2.95 ± 0.13 ^g	8.37 ± 0.36 ^f	0.27 ± 0.01 ^f	1.42 ± 0.02 ^d	80.00 ± 3.54 ^c
	1	35.15 ± 0.25	38.55 ± 0.32 ^f	14.00 ± 0.11	3.40 ± 0.17 ^f	9.67 ± 0.48 ^f	0.31 ± 0.01 ^f	1.41 ± 0.02 ^d	91.25 ± 2.39 ^b
	3	35.30 ± 0.19	41.58 ± 0.33 ^e	14.08 ± 0.07	6.28 ± 0.14 ^e	17.77 ± 0.32 ^e	0.55 ± 0.01 ^e	1.49 ± 0.01 ^c	100.00 ± 0.00 ^a
24	0	35.10 ± 0.09	43.18 ± 0.27 ^{cd}	14.03 ± 0.09	8.08 ± 0.18 ^c	23.01 ± 0.46 ^c	0.69 ± 0.01 ^c	1.57 ± 0.02 ^b	100.00 ± 0.00 ^a
	1	35.18 ± 0.18	43.50 ± 0.23 ^c	14.13 ± 0.09	8.33 ± 0.09 ^c	23.67 ± 0.22 ^c	0.71 ± 0.01 ^c	1.55 ± 0.02 ^{bc}	100.00 ± 0.00 ^a
	3	35.28 ± 0.30	47.53 ± 0.27 ^a	14.13 ± 0.09	12.25 ± 0.06 ^a	34.74 ± 0.41 ^a	0.99 ± 0.01 ^a	1.69 ± 0.02 ^a	100.00 ± 0.00 ^a
32	0	35.20 ± 0.34	41.65 ± 0.40 ^e	14.03 ± 0.09	6.45 ± 0.20 ^e	18.33 ± 0.61 ^e	0.56 ± 0.02 ^e	1.51 ± 0.02 ^{bc}	100.00 ± 0.00 ^a
	1	35.23 ± 0.36	42.58 ± 0.24 ^d	14.05 ± 0.06	7.35 ± 0.19 ^d	20.89 ± 0.72 ^d	0.63 ± 0.02 ^d	1.54 ± 0.01 ^{bc}	100.00 ± 0.00 ^a
	3	35.40 ± 0.30	44.63 ± 0.25 ^b	13.98 ± 0.09	9.23 ± 0.13 ^b	26.07 ± 0.53 ^b	0.77 ± 0.02 ^b	1.64 ± 0.02 ^a	100.00 ± 0.00 ^a
Water temperature effect									
18		35.23 ± 0.11	35.23 ± 0.11	14.00 ± 0.06	4.21 ± 0.45 ^c	11.94 ± 1.27 ^c	0.37 ± 0.04 ^c	1.44 ± 0.02 ^c	90.42 ± 2.78 ^b
24		35.18 ± 0.11	35.18 ± 0.11	14.09 ± 0.05	9.55 ± 0.58 ^a	27.14 ± 1.63 ^a	0.80 ± 0.04 ^a	1.60 ± 0.02 ^a	100.00 ± 0.00 ^a
32		35.28 ± 0.18	35.28 ± 0.18	14.02 ± 0.04	7.68 ± 0.36 ^b	21.76 ± 1.02 ^b	0.65 ± 0.03 ^b	1.56 ± 0.02 ^b	100.00 ± 0.00 ^a
DRLM level effect									
	0	35.18 ± 0.13	41.00 ± 0.65 ^c	13.99 ± 0.05	5.83 ± 0.65 ^c	16.57 ± 1.86 ^c	0.51 ± 0.05 ^c	1.50 ± 0.02 ^b	93.33 ± 3.04 ^c
	1	35.18 ± 0.14	41.54 ± 0.66 ^b	14.06 ± 0.05	6.36 ± 0.65 ^b	18.08 ± 1.84 ^b	0.55 ± 0.05 ^b	1.50 ± 0.02 ^b	97.08 ± 1.44 ^b
	3	35.33 ± 0.14	44.58 ± 0.75 ^a	14.06 ± 0.05	9.25 ± 0.74 ^a	26.19 ± 2.10 ^a	0.77 ± 0.06 ^a	1.60 ± 0.03 ^a	100.00 ± 0.00 ^a
Two-way ANOVA: P-values									
Interaction		0.998	0.032	0.819	<0.001	<0.001	<0.001	0.433	<0.001
Water temperature		0.911	<0.001	0.412	<0.001	<0.001	<0.001	<0.001	<0.001
DRLM level		0.732	<0.001	0.572	<0.001	<0.001	<0.001	<0.001	<0.001

Means ± SE are presented for each parameter. Means in the same column with different superscripts are significantly different ($P < 0.05$).

^a Treatment means represent the average values of three aquaria per treatment.

diet and exposed to 18°C. Group 2 (G2) fed on DRLM 1% and exposed to 18°C. Group 3 (G3) fed on DRLM 3% and exposed to 18°C. Group 4 (G4) fed on basal diet and exposed to 24°C. Group 5 (G5) fed on DRLM 1% and exposed to 24°C. Group 6 (G6) fed on DRLM 3% and exposed to 24°C. Group 7 (G7) fed on basal diet and exposed to 32°C. Group 8 (G8) fed on DRLM 1% and exposed to 32°C. Group 9 (G9) fed on DRLM 3% and exposed to 32°C. The exposure continued for 30 days.

2.4. Growth performance evaluation

After the feeding trial, the fish of every group were weighed to record final body weight (FBW). Fish weight gain (WG) was calculated by getting the difference between FBW and IBW. Average daily gain (ADG) was calculated by subtracting WG on the number of trial days. The specific growth rate (SGR), body weight gain percentage (BWG %) and survival rate (SR) (Jobling, 1994) and condition factor (K) (Froese, 2006) were determined according to the following formulas:

$$SGR = (\ln FBW - \ln IBW) / \text{time intervals(days)} \times 100.$$

$$BWG \% = (FBW - IBW) / IBW \times 100.$$

$$SR = (\text{the final number of fish} / \text{the initial number of fish}) \times 100.$$

$$K = (W/L^3) \times 100; W: \text{wet weight(g)} \text{ and } L: \text{length(cm)}.$$

2.5. Sampling

After 15 and 30 days of the experiment, blood samples were collected from each group by puncturing the caudal blood vessels samples. The blood collected samples were centrifuged for 15 min at 1075 × g for serum separation and were then stored at -20°C until analysis of lysozyme activity, immunoglobulin M (IgM), and cortisol levels. Then, fish were dissected immediately and liver tissues were taken, homogenized in 10 vol of phosphate buffer saline (pH 7.4) then the homogenates were centrifuged for 30 min at 664 × g at 4°C and the supernatant kept at -80°C till evaluation of oxidant/antioxidant status. Also, small portions of the liver were immediately preserved in a liquid nitrogen container and subsequently stored at -80 °C for real-time PCR analysis.

2.6. Biochemical parameters estimation

Selective oxidative markers were assessed using colorimetric commercial kits purchased from Biodiagnostic Co. (Cairo, Egypt). Catalase enzyme (CAT) was measured following the procedure of Aebi (1984). SOD was measured by the method of Nishikimi et al. (1972). MDA was evaluated according to the method of Uchiyama and Mihara (1978). An enzyme-linked immunosorbent assay (ELISA) kit was used for measuring the serum level of IgM, according to the manufacturer's

Table 4

Lipid peroxidation, immune response, and cortisol level of Nile tilapia fed diets containing three levels of dried rocket leaves meal (DRLM) and reared at 18, 24, and 32°C after 15 days of the experiment.

Diets		MDA nmol/g tissue	Lysozyme µg/ml	IgM ng/ml	Cortisol ng/ml
Water temperature °C	DRLM %				
Individual treatment means ^a					
18	0	1.34 ± 0.05 ^a	4.64 ± 0.20 ^e	9.00 ± 1.73 ^e	10.08 ± 0.08 ^e
	1	0.93 ± 0.03 ^b	12.34 ± 0.47 ^{cd}	12.00 ± 1.15 ^{de}	11.20 ± 0.17 ^d
	3	0.89 ± 0.06 ^b	13.98 ± 0.27 ^c	15.00 ± 1.15 ^{cd}	13.51 ± 0.50 ^c
24	0	0.24 ± 0.02 ^{cd}	11.60 ± 0.18 ^{cd}	18.00 ± 1.15 ^c	14.97 ± 0.18 ^b
	1	0.15 ± 0.02 ^d	13.00 ± 2.31 ^{cd}	36.00 ± 1.73 ^a	13.66 ± 0.33 ^c
	3	0.17 ± 0.03 ^d	26.00 ± 1.73 ^a	40.00 ± 0.58 ^a	14.03 ± 0.30 ^c
32	0	0.96 ± 0.02 ^b	6.78 ± 0.46 ^e	29.00 ± 0.58 ^b	16.60 ± 0.28 ^a
	1	0.34 ± 0.08 ^c	10.00 ± 1.15 ^d	32.00 ± 1.15 ^b	14.26 ± 0.16 ^{bc}
	3	0.18 ± 0.05 ^d	18.70 ± 0.42 ^b	39.00 ± 1.73 ^a	14.00 ± 0.37 ^c
Water temperature effect					
18		1.05 ± 0.08 ^a	10.32 ± 1.45 ^b	12.00 ± 1.11 ^b	11.60 ± 0.53 ^c
24		0.19 ± 0.02 ^c	16.87 ± 2.44 ^a	31.33 ± 3.44 ^a	14.22 ± 0.24 ^b
32		0.49 ± 0.12 ^b	11.83 ± 1.82 ^b	33.33 ± 1.61 ^a	14.95 ± 0.44 ^a
DRLM level effect					
	0	0.85 ± 0.16 ^a	7.67 ± 1.04 ^c	18.67 ± 2.96 ^c	13.88 ± 0.98 ^a
	1	0.47 ± 0.12 ^b	11.78 ± 0.88 ^b	26.67 ± 3.77 ^b	13.04 ± 0.48 ^b
	3	0.41 ± 0.12 ^b	19.56 ± 1.82 ^a	31.33 ± 4.13 ^a	13.85 ± 0.22 ^a
Two-way ANOVA: <i>P</i> -values					
Interaction		<0.001	0.001	<0.001	<0.001
Water temperature		<0.001	<0.001	<0.001	<0.001
DRLM level		<0.001	<0.001	<0.001	0.003

Means ± SE are presented for each parameter. Means in the same column with different superscripts are significantly different ($P < 0.05$).

^a Treatment means represent the average values of three aquaria per treatment. MDA: malondialdehyde; IgM: immunoglobulin M.

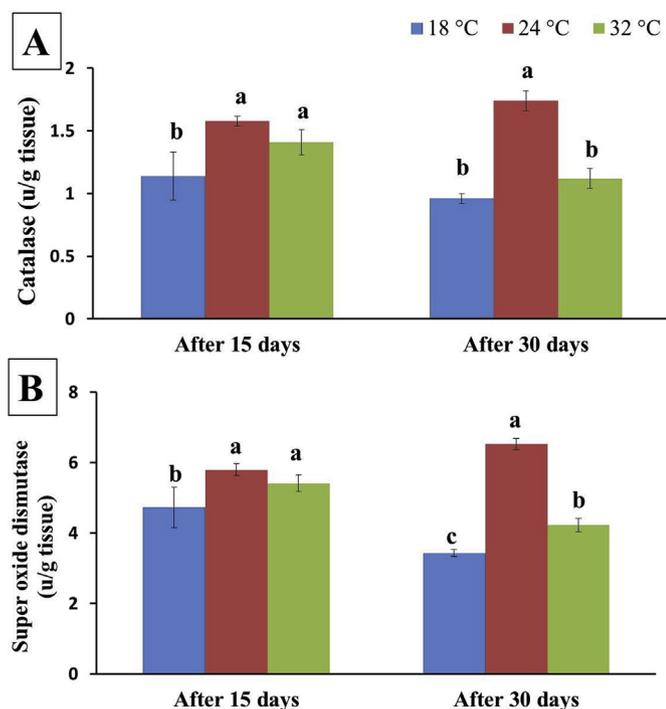


Fig. 1. Effect of different water temperature (18, 24, and 32°C) on catalase (A) and super oxide dismutase (B) activity in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean ± SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

instructions. Lysozyme activity was measured with spectrophotometry following the method of Ellis (1990). Using radioimmunoassay, cortisol concentration was measured as validated before by Chiu et al. (2003).

2.7. Expression of IL-1 β , CAT, and SOD by quantitative RT (real-time)-PCR from liver tissue

Total RNA was isolated from three hepatic tissue samples from each group after 15 and 30 days of the trial by easy-REDTM according to the protocol of the manufacturer (iNtRON Biotechnology, South Korea). The complementary DNA was synthesized in line with the manufacturer's guidelines of a Quantitect[®] Reverse Transcription kit (Qiagen, Germany). The quantitative real-time PCR analysis was done using SYBR green PCR master mix (StepOnePlus, Applied Biosystem, USA). The thermal profile for real-time PCR and primer sequences are revealed in Table 2. To assess the gene expression variation in the different samples, the CT of each sample was matched with that of the positive control group in relation to the " $\Delta\Delta Ct$ " method (Livak and Schmittgen, 2001).

2.8. Statistical analyses

The obtained results were statistically analyzed by factorial experiment using SPSS 21.0 for Windows according to the following Model: $Y_{ijk} = \mu + W_i + L_j + WL_{ij} + e_{ijk}$, where Y_{ijk} = an observation, μ = the overall mean, W_i = the fixed effect of the water temperature, L_j = the fixed effect of the DRLM supplementation level, WL_{ij} = the interaction between the water temperature and DRLM supplementation level, and e_{ijk} = random error.

If significant interaction between water temperature and DRLM supplementation level were found, the results were analyzed by one-way ANOVA followed by Duncan's multiple range tests to examine the impact of DRLM supplementation level at each water temperature. If only the main effects of the current study (DRLM supplementation level or water temperature) were significant, the data were analyzed by the general linear model procedure followed by Duncan's multiple range tests to inspect only the main effects. Data are presented as means plus or minus the standard error. $P < 0.05$ was fixed as the significance minimum level.

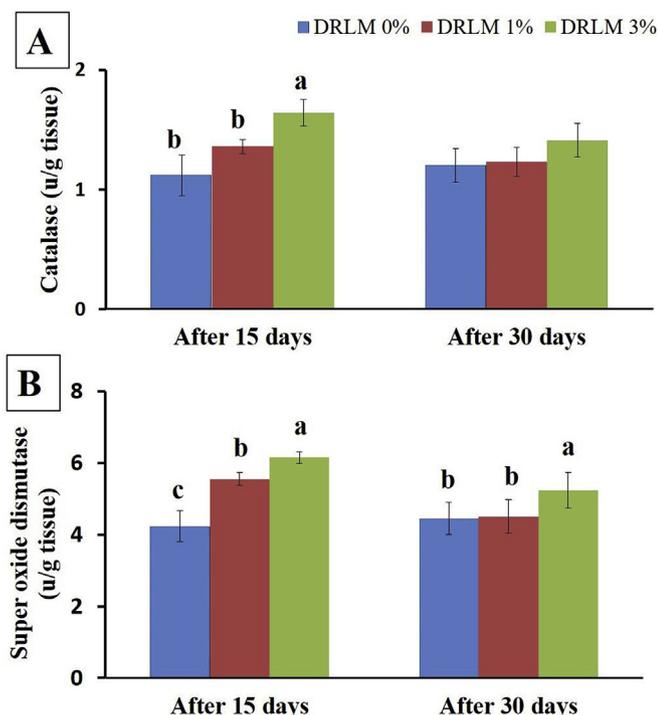


Fig. 2. Effect of dried rocket leaves meal (DRLM) supplementation (0, 1, and 3%) on catalase (A) and super oxide dismutase (B) activity in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean ± SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

3. Results

3.1. Clinical observation

Initially, fish that reared at 24°C and fed on 0, 1, or 3% DRLM

showed a normal appetite and normal swimming behavior throughout the experimental period. In contrast, fish groups that exposed to 18°C and fed on the control diet (0% DRLM) suffered from anorexia, lethargy, and decrease swimming behavior. In contrast, there was an increase in the swimming behavior in fish that fed on 1 and 3% DRLM and reared at 18°C. Fish group that exposed to 32°C and fed on basal diet with 0% DRLM showed darkness of the body surface and an increase in the swimming behavior, while these signs were notably reduced in groups that fed 1 and 3% DRLM.

3.2. Effects on growth performance

Table 3 demonstrates the effects of dietary supplementation of different DRLM levels on tilapia that reared at three different temperatures for 30 days on growth performance indicators, survival rate, and somatic indices. Initially, no significant change in fish length was recorded in different DRLM supplemented groups at the various water temperatures. However, a significant reduction of FBW, WG, DWG%, SGR, CF, and SR was apparent when fish was reared at 18°C compared to those reared at 24°C or 32°C. Nonetheless, there was a significant increase in the former indicators in fish that reared at 24°C compared to those reared at 32°C. There was a dose-dependent increase in all growth performance indicators together with condition factor and survival rate by increasing DRLM addition level with the best performance with 3% DRLM dietary level. There was a significant interaction between DRLM dietary supplementation and water temperature on FBW, WG, DWG%, SGR, and SR. Fish that fed diet supplemented with 3% DRLM and reared at 18°C or 24°C and those fed diet supplemented with 1 or 3% DRLM and reared at 32°C showed a significant increment in FBW, WG, DWG%, SGR, and CF. Additionally, supplementation of fish that reared at 18°C with 1% DRLM or 3% DRLM evoked a significant increase in SR.

3.3. Effects on biochemical parameters after 15 days of the experiment

Table 4 and Figs. 1–3 showed the change in oxidative stress parameters, immunity indicators and stress hormones levels of tilapia reared

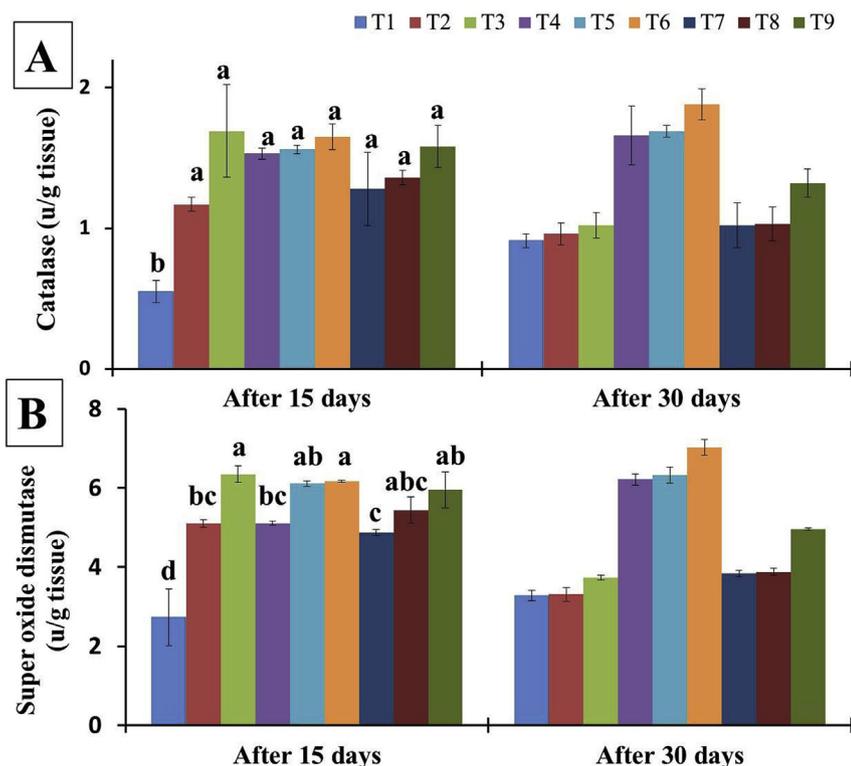


Fig. 3. Effect of the interaction between different water temperature (18, 24, and 32°C) and dried rocket leaves meal (DRLM) supplementation (0, 1, and 3%) on catalase (A) and super oxide dismutase (B) activity in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean ± SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

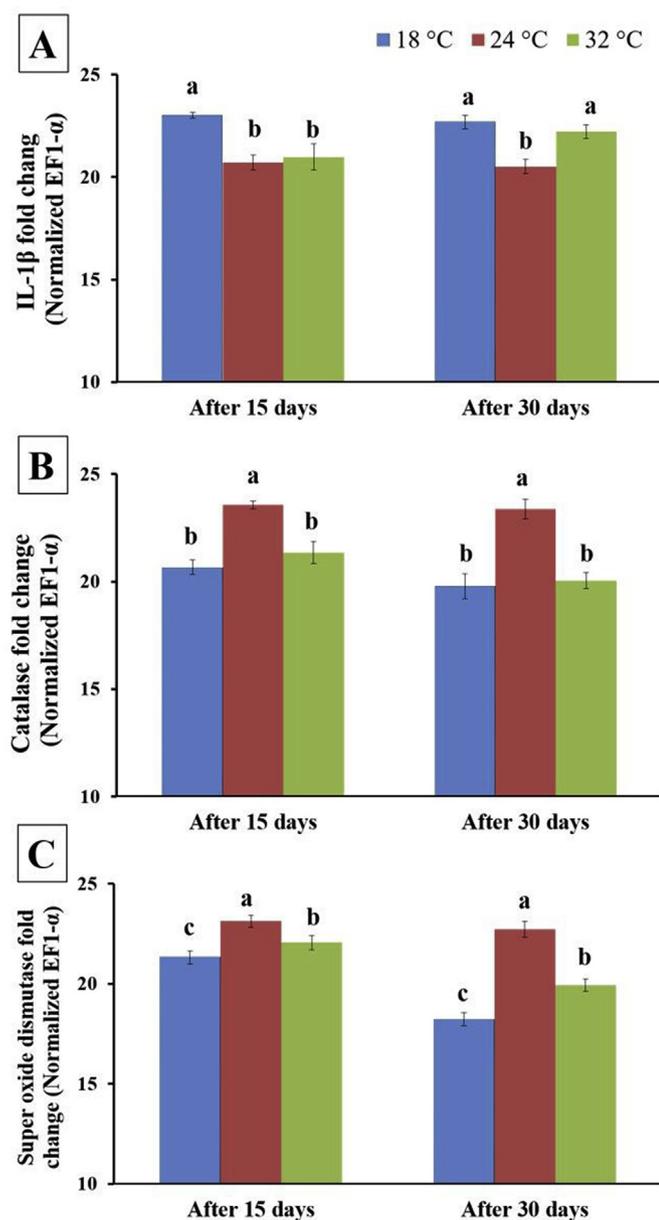


Fig. 4. Effect of different water temperature (18, 24, and 32°C) on interleukin 1 β (A), catalase (B), and super oxide dismutase expression (C) in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean \pm SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

at three different temperatures and supplemented with different levels of DRLM after 15 days of the experiment. Fish that reared at 18°C displayed a significant depression of SOD, CAT, and lysozymes activities together with a significant reduction of IgM level with a significant increment in MDA and cortisol level compared to those cultured at 24°C or 32°C. The DRLM (1% and 3%) dietary supplementation significantly enhanced SOD, IgM, and lysozymes levels with significantly suppressed MDA level compared to the non-supplemented groups. The CAT level was significantly increased in fish that fed 3% DRLM compared to other addition levels. A significant decline in cortisol concentration in fish that fed 1% DRLM was noted compared to other addition levels.

There was a significant interaction between DRLM dietary supplementation and water temperature on oxidative stress and immunity parameters. Supplementation of fish that reared at 18°C with 1% DRLM or 3% DRLM in their diet evoked a significant increase in SOD, CAT,

cortisol, and lysozymes activities with a significant decline in MDA and cortisol levels. Fish that fed diet fortified with 3% DRLM and reared at 24°C showed a significant increment in SOD and lysozyme activity. Also, the fish that fed diet supplemented with 1% or 3% DRLM and reared at 24°C showed a significant increase in CAT activity with a reduced cortisol level. The addition of 1% or 3% DRLM to the diet of fish that reared at 32°C significantly reduced MDA and cortisol levels with increased lysozyme activity compared to the non-supplemented group at the same temperature. Also, both IgM and SOD levels were significantly elevated in the fish group that reared at 32°C and supplemented with 3% DRLM in their diet.

3.4. Effects on biochemical parameters after 30 days of the experiment

The effect of 30-day dietary supplementation of different DRLM levels on oxidative stress parameters, immunity indicators, and stress hormones levels in tilapia reared at three different temperatures are shown in Table 5. Rearing of fish at 18°C significantly decreased SOD, CAT, IgM, cortisol, and lysozymes activities with a significant rise in MDA level was observed compared to rearing at 24°C. Rearing of fish at 32°C significantly decreased SOD, CAT, IgM, and lysozymes activities with a significant rise in MDA and cortisol levels was recorded compared to rearing at 24°C. Addition of 1% or 3% DRLM to fish diet significantly enhanced lysozymes activity and reduced MDA level. Also, a significant improvement in SOD and IgM concentration was evident with adding 3% DRLM to fish diet.

At the end of the trial, no significant interaction was recorded between DRLM dietary supplementation and water temperature on SOD, CAT, and MDA levels. However, the lysozyme activity was significantly increased with 1% or 3% DRLM supplementation to fish diet at all tested water temperatures. The IgM level was significantly improved in fish that reared at 18°C and 24°C with 3% DRLM supplementation. Fish that fed diet fortified with 3% DRLM and reared at 18°C showed a significant increase in cortisol level. At 32°C, the addition of 1% or 3% DRLM significantly reduced cortisol level.

3.5. Effects oxidative stress and immune-related genes

Fig. 4A showed significantly up-regulated immune-related gene expression (IL-1 β) in the livers of *O. niloticus* that reared at 18°C for 15 successive days (23.01 ± 0.15) compared with those reared at 24°C (20.71 ± 0.37). Also, exposing fish to 18°C and 32°C for 30 consecutive days significantly up-regulated IL-1 β expression (22.68 ± 0.33 and 22.22 ± 0.33 , respectively) relative to those reared at 24°C (20.51 ± 0.35). As demonstrated in Fig. 4B, after both 15 and 30 days of feeding trials, a significant down-regulation of CAT expression in fish reared at 18°C (20.66 ± 0.34 and 19.78 ± 0.59 , respectively) and 32°C (21.35 ± 0.52 and 20.05 ± 0.37 , respectively) compared with those reared at 24°C (23.57 ± 0.18 and 23.37 ± 0.45 , respectively). Likewise, after both 15 and 30 days of the experiment, a significant down-regulation of SOD expression in fish reared at 18°C (21.31 ± 0.33 and 18.21 ± 0.33 , respectively) and 32°C (22.05 ± 0.35 and 19.93 ± 0.32 , respectively) compared with those reared at 24°C (23.12 ± 0.29 and 22.72 ± 0.39 , respectively) Fig. 4C.

As shown in Fig. 5A, the addition of 1% or 3% DRLM for 15 days and 3% DRLM for 30 days significantly down-regulated IL-1 β gene expression compared to the non-supplemented group. Notably, the maximum reduction was achieved with the addition of 3% DRLM at both experimental period durations. In contrast, CAT and SOD genes expression were significantly increased with the addition of DRLM at both 15 and 30 days of the experiment Fig. 5B and C.

As displayed in Fig. 6A, at 15 days of the experiment, the addition of 1% or 3% DRLM in fish that reared at 24°C or 32°C evoked a significant downregulation of IL-1 β gene expression compared to the non-supplemented group. Additions of 1% or 3% DRLM have not significantly affect IL-1 β gene expression in fish reared at 18°C. Nevertheless, the

Table 5

Lipid peroxidation, immune response, and cortisol level of Nile tilapia fed diets containing three levels of dried rocket leaves meal (DRLM) and reared at 18, 24, and 32°C after 30 days of the experiment.

Diets		MDA nmol/g tissue	Lysozyme µg/ml	IgM ng/ml	Cortisol ng/ml
Water temperature °C	DRLM %				
Individual treatment means ^a					
18	0	1.55 ± 0.11	4.00 ± 0.23 ^g	14.00 ± 0.58 ^c	12.00 ± 0.58 ^f
	1	1.13 ± 0.06	6.50 ± 0.28 ^{ef}	16.00 ± 0.58 ^c	12.86 ± 0.77 ^{ef}
	3	0.89 ± 0.05	9.12 ± 0.19 ^c	19.33 ± 0.88 ^b	14.25 ± 0.44 ^{de}
24	0	0.78 ± 0.03	9.89 ± 0.51 ^c	21.33 ± 0.88 ^b	15.96 ± 0.42 ^{cd}
	1	0.65 ± 0.03	10.97 ± 0.41 ^b	22.00 ± 1.15 ^b	15.76 ± 0.21 ^{cd}
	3	0.49 ± 0.04	16.57 ± 0.32 ^a	34.00 ± 1.73 ^a	15.25 ± 0.14 ^d
32	0	1.31 ± 0.15	6.08 ± 0.05 ^f	20.00 ± 0.58 ^b	20.30 ± 0.96 ^a
	1	1.06 ± 0.05	7.13 ± 0.08 ^{de}	21.00 ± 0.58 ^b	18.50 ± 0.50 ^b
	3	0.87 ± 0.05	7.66 ± 0.39 ^d	21.67 ± 0.88 ^b	17.36 ± .26 ^{bc}
Water temperature effect					
18		1.19 ± 0.10 ^a	6.54 ± 0.75 ^b	16.44 ± 0.85 ^c	13.04 ± 0.45 ^c
24		0.64 ± 0.04 ^b	12.48 ± 1.06 ^a	25.78 ± 2.16 ^a	15.66 ± 0.18 ^b
32		1.08 ± 0.08 ^a	6.96 ± 0.26 ^b	20.89 ± 0.42 ^b	18.72 ± 0.54 ^a
DRLM level effect					
	0	1.21 ± 0.13 ^a	6.66 ± 0.88 ^c	18.44 ± 1.18 ^b	16.09 ± 1.25
	1	0.94 ± 0.08 ^b	8.20 ± 0.71 ^b	19.67 ± 1.01 ^b	15.71 ± 0.86
	3	0.75 ± 0.07 ^c	11.12 ± 1.39 ^a	25.00 ± 2.36 ^a	15.62 ± 0.48
Two-way ANOVA: <i>P</i> -values					
Interaction		0.188	<0.001	<0.001	0.003
Water temperature		<0.001	<0.001	<0.001	<0.001
DRLM level		<0.001	<0.001	<0.001	0.538

Means ± SE are presented for each parameter. Means in the same column with different superscripts are significantly different ($P < 0.05$).

^a Treatment means represent the average values of three aquaria per treatment. MDA: malondialdehyde; IgM: immunoglobulin M.

interaction between the exposure to different temperatures and DRLM addition on CAT and SOD genes expression at 15 days of the experiment and IL-1 β , CAT, and SOD genes expression at 30 days of the experiment Fig. 6A–C.

4. Discussion

In poikilothermic organisms, changes in water temperature could result in significantly retarded growth rates (Ma et al., 2015). This was apparent in the current study in fish that reared at either 18°C or 32°C with adequate growth rate was evident in those reared at 24°C. The former water temperature falls within the appropriate temperature range, 25–30°C, reported by El-Sherif and El-Feky (2009). Elliott (1972) elucidated that higher temperatures stimulate evacuation of gastric content resulting in poor feed utilization and suppressed growth. Azaza et al. (2008) verified that such growth retardation is owed to the higher energy consumed for metabolic maintenance and occurs mainly due to appetite loss when the temperature reaches the maximum extreme of the tolerance range. Also, heat or cold thermal stress has been found to reduce growth performance, possibly due to extreme reactive oxygen species (ROS) that oxidize and damage cellular biological molecules, hinder some ATPases activities and finally induce various injuries to intestinal tissues and impaired growth and feed utilization (Al-Sagheer et al., 2017; Blagojevic et al., 2011; Daader et al., 2018).

Accordingly, it could be proposed that such enhancements in growth due to DRLM supplementation even in heat or cold stressed fish could be chiefly linked to its antioxidant activity (Villatoro-Pulido et al., 2012). Several bioactive with strong antioxidant activity have been previously identified in the chromatographic profile of *E.sativa* leaves like gallic acid, kaempferol, and quercetin (Grami et al., 2018). In addition, antibacterial, antiprotozoal and antifungal of DRLM could be a probable mechanism of the enhanced growth (Leung and Foster, 1996). Also, the DRLM immunomodulating effect could partly elucidate its positive effects on fish growth. Similar growth-enhancing effects of DRLM have been previously reported in red tilapia (Abd Elmonem et al., 2002), and Nile tilapia (Khalil et al., 2015; Mahmoud et al., 2009).

Under heat or cold stress conditions, an ample of ROS is released leading to oxidative damage of macromolecules (Heise et al., 2006). Moreover, high levels of corticosteroids and catecholamines are released resulting in lipid peroxidative impairment (Bahrami et al., 2012). Therefore, in the existing study, to elucidate the core mechanism of DRLM in mitigating temperature stress impacts in Nile tilapia, antioxidants and lipid peroxidation indicators were determined. Primarily, a sharp drop in SOD and CAT activities with a significant down-regulation of their encoding genes with a sharp increment in the MDA level was evident in fish reared at either 18°C or 32°C owing to temperature stress-induced oxidative stress and lipid peroxidation. The decline of the antioxidant enzymes' activities could be because of their depletion in the course of fighting the generated ROS to preserve the steady-state amount of generated free radicals (Belhadj Slimen et al., 2016; Mohamed et al., 2016). In contrast, heat-stressed Nile tilapia fed diets supplemented with 1 or 3% DRLM had higher concentrations of SOD and CAT indicating a notable improvement of their oxidative status. These favorable results could be highly linked to antioxidant constituents of DRLM including vitamin C, carotenoids, flavonoids, glucosinolates, and volatile oils (Barillari et al., 2005; Hanafi et al., 2010). In particular, glucosinolates were reported to potentially capable of protecting cells against oxidative stress through the initiation of phase II enzymes, reducing hydrogen peroxide and alkyl hydroperoxides present in cells and acting as a precursor of sulforaphane, a powerful inducer detoxifying electrophiles and augment cellular antioxidant fortifications (Kim et al., 2004).

A strong correlation has been documented between the water temperature and the immune status of fish (Dittmar et al., 2014). For instance, Dominguez et al. (2004) reported that IgM in fishes is the only element of the humoral defense system that is influenced by environmental temperature and suggested that some species may have an optimal thermal range for immune function. Herein, a significant reduction of lysozyme activity together with the depletion of IgM content was obvious in fish reared at 18°C or 32°C. A similar variation of IgM level has been reported in certain fishes reared at different temperatures (Bao et al., 2018; Magnadóttir et al., 1999). Furthermore, in this study, we evaluated the effect of temperature stress on the mRNA expression of a

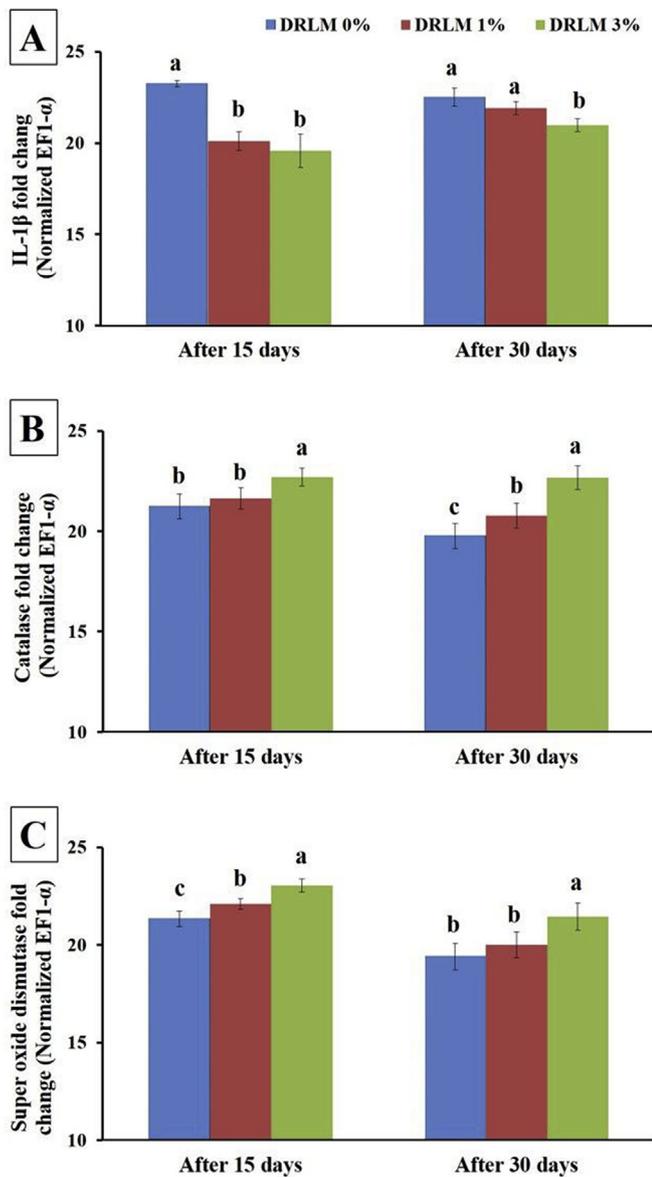


Fig. 5. Effect of dried rocket leaves meal (DRLM) supplementation (0, 1, and 3%) on interleukin 1 β (A), catalase (B), and super oxide dismutase expression (C) in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean \pm SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

very important immune-related gene produced by leukocytes, IL-1 β . IL-1 β has been reported to play a vital immunological role during temperature conditions (Pérez-Casanova et al., 2008). Herein, a significant upregulation of IL-1 β was recorded in fish reared at 18° C or 32°C. Variation in the transcription of IL-1 β cytokines in fish following exposure to variable temperatures was previously reported (Basu et al., 2015; Polinski et al., 2013). Interestingly, incorporation of DRLM in thermally stress fish significantly counteracted the immunosuppression state. This could be linked to the immunostimulant ingredients of DRLM. For instance, DRLM has been reported to contain Zn, Cu, Fe, Mg, Mn and other elements with known immunostimulatory activities (Abdo and Zeinab, 2003). Also, DRLM has been known as a good source of vitamin A with a vital role for normal growth, healthy mucous membranes, and immune status (Kim et al., 2004). A similar immunostimulant effect has been previously reported in Nile tilapia reared under non stressful conditions (Khalil et al., 2015).

Cortisol level is one of the most common stress indicators in fish

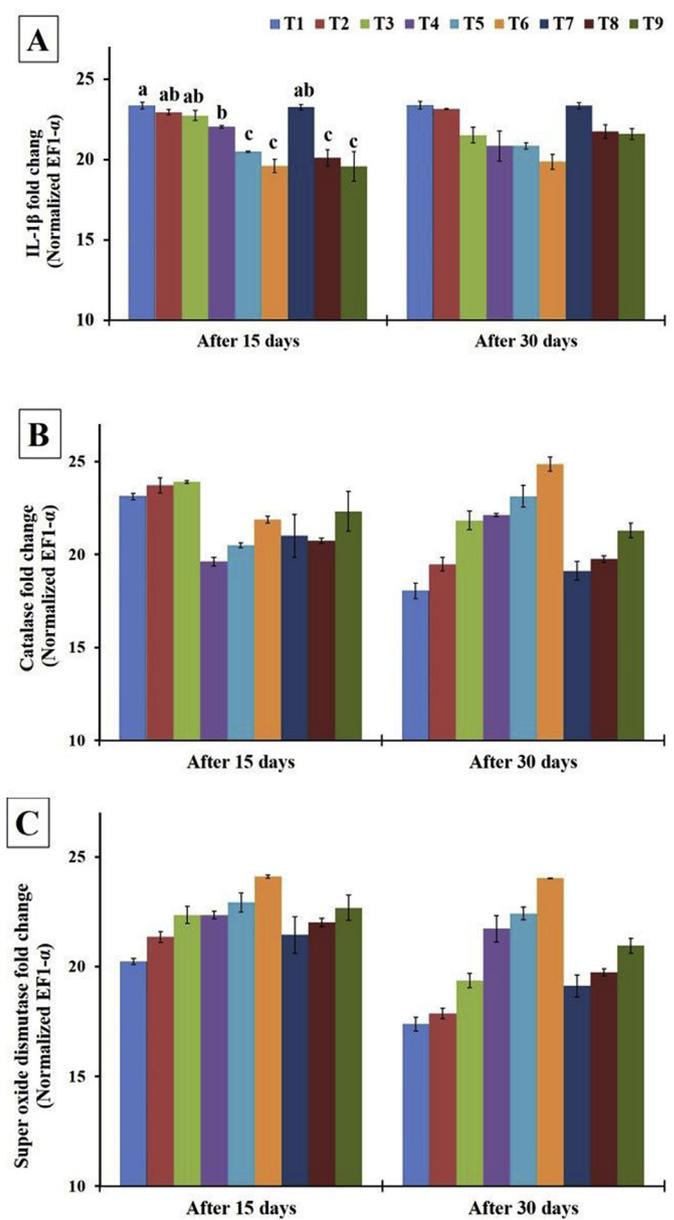


Fig. 6. Effect of the interaction between different water temperature (18, 24, and 32°C) and dried rocket leaves meal (DRLM) supplementation (0, 1, and 3%) on interleukin 1 β (A), catalase (B), and super oxide dismutase expression (C) in Nile tilapia after 15 and 30 days of the experiment. Data expressed as mean \pm SE, n = 3 for each group. Each bar carrying different letters (a, b, and c) was significantly different.

(Panase et al., 2018). The rise of this stress hormone modulates diverse immune parameters of fish including reduction of the primary immune response of lymphocytes (Carlson et al., 1993), respiratory burst activity (Esteban et al., 2004), circulating IgM levels (Cuesta et al., 2006), and IL-1 β expression (Zou et al., 2000). The obtained results in the current study revealed that after 15 days of the experiment, 18°C exposed fish had lower cortisol level which could be attributed to lower synthesis and secretion of cortisol due to minor metabolic rate. A similar result obtained by Davis (2004) in sunshine bass. A significantly elevated level of cortisol was recorded in fish reared at 32°C reflecting the stress status of the experimental fish. Similar result obtained by Delaney et al. (2005) in *O. niloticus* and Musa et al. (2017) in Red Hybrid Tilapia. In contrast, incorporation of DRLM in the diet reduces the cortisol level in the groups reared at 24°C and 32°C after 15 and 30 days of the experiment. This may be attributed to vitamin C content of

DRLM as vitamin C has been reported to inhibit the transformation of unsaturated fatty acids into cholesterol esters, which are vital components of cortisol (Peters et al., 2001). Also, the antioxidant activity of DRLM could be another possible reason (Maia et al., 2015).

5. Conclusion

From the obtained results in the current study, it could be concluded that during heat or cold stress condition, DRLM dietary supplements offer an easily applicable additive for improving growth performance, lipid peroxidation, the immune, and antioxidative status of Nile tilapia without plausible side effects. Further studies are required to assess the effects of DRLM as natural growth promoter under other stressful conditions for other fish species.

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