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Research Article

Effect of curcumin on growth performance and antioxidant stress status of Nile tilapia (*Oreochromis niloticus*)

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Abstract

In the present study, the anti-oxidative effects of dietary curcumin (CUR) were evaluated in Nile tilapia (*Oreochromis niloticus*) challenged with cold temperature condition in floating cages system. Fish with an average weight of 22±0.5 g were divided into four groups and fed daily with free basal diet (control); 1, 2, and 3% CUR for a five-week period. Oxidative status and growth parameters were measured. Results indicated that CUR supplementation markedly enhanced antioxidative status which was noticed by enhanced superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidase activities. In addition, improvement in growth performance including body mass gain, specific growth rate, condition factor and feed conversion ratio were noticed. The expression of related HSP70, *IL-1β* and CC5 were markedly up-regulated over the control. Conclusively, dietary CUR markedly enhanced anti-oxidative status all over experimental period, proposing its usage as natural anti-cold stress supplement and thus could increase time spent in outdoor culture systems through improving tolerance to cold water temperature.

Keywords: Curcumin, Growth, Antioxidants, Cold stress, *Oreochromis niloticus*

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Introduction

Cage culture is one of the most convenient aquaculture systems due to availability and using already existing water sources (Jiang, 2016) and it is a rapidly developing fish farming system in Egypt (Kleih *et al.*, 2013). Cage culture faces many environmental stressful conditions among which temperature changes are considered as stressful condition that can affect all biological mechanisms (Bly and Clem, 1992; Bowden, 2008) as well as all living organisms, especially in ectothermic animals (Lushchak, 2011). Those temperature fluctuations and changes can easily cause oxidative responses (Lushchak and Bagnyukova, 2006), which are accompanied with release of reactive oxygen species (ROS), as superoxide anion, hydrogen peroxide and hydroxyl radicals (Livingstone, 2001). Antioxidant mechanisms include specific antioxidants as, superoxide dismutase, catalase and glutathione (Cossu *et al.*, 1997) and stress proteins, such as molecular heat shock proteins (Lushchak and Bagnyukova, 2006). At the cellular level, heat shock protein 70 is the principle gene responsible for preventing apoptosis and oxidative damage (Beere *et al.*, 2000).

Many natural herbal plants have anti-oxidative properties and looks like SOD in their effect. Curcumin (CUR) is the key active ingredient of *Curcuma_longa* and has different medicinal properties including anti-oxidative (Boonla *et al.*, 2014; Enis Yonar *et al.*, 2019), anti-stress (Jiang, 2016), anti-inflammatory and immunomodulatory effects (Mandal *et al.*, 2009). CUR incorporation in carp diets showed enhancement in growth, digestion and antioxidants responses (Jiang, 2016).

Information about the effect of CUR incorporation on tilapia growth and the expression of antioxidant genes (HSP70, *IL-1β* and C5) is still scarce. Current study aimed to evaluate the effect of CUR on growth and oxidative stress status in *Oreochromis niloticus* held in floating cages system and exposed to natural cold water temperature conditions.

Materials and methods

Fish

O. niloticus weighting 22±0.5 g were obtained from private fish farm at Kafr El Sheikh Governorate, Egypt, transferred to an in-water floating cages system and kept in large 20,000 L floating Hapa at Rasheed, Behera, Egypt. Acclimation took ten days feeding basal diet and water parameters were checked daily all over the experimental period.

Curcumin (CUR)

CUR was a commercial product in powder form provided by Organic Herb Inc., China.

Diets preparation

Four diets were formulated, by dividing basal diet (Crude Protein 32.32%, Crude Fat 50 g/kg, Crude Fiber 11.49%, Calcium 15 g/kg, Phosphorous (P) 3 g/kg and Ash Maximum 5.52 %) into four parts and supplemented with 0, 1, 2,

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and 3% CUR/kg feed. All ingredients were mixed and blended to obtain the desired experimental concentrations. After drying, diets were stored at −20°C.

Experimental design

Fish were divided onto four groups each in three replicates in outdoor 500-L floating cages (90 fish/ group, 30 fish/ replicate) that were held in a large 20,000-L Hapa and fed with 0 (control), 1, 2, and 3% CUR incorporated diets. Fish were fed to satiation twice daily for five weeks. Average dissolved oxygen based on daily measurement was 8.0±0.6 mg/L and range of water temperature was 2.0 to 13.5°C; averaging 9.0±0.5°C. This temperature is lower than its optimal level (24-32°C) (El-Sayed and Kawanna, 2008). This level was challenging and not lethal, depending on several factors (feeding, genetic, season, condition of the fish and environment) (Atwood *et al.*, 2003).

Sampling

Liver samples were collected in PBS (pH 7.4, kept at –20°C) for antioxidants assays and in RNAlater (Ambion, USA), kept at –80°C) for gene expression study.

Growth parameters

All fish were weighed at the beginning of the experiment and at week 5 and growth parameters were measured using formulas described by Elabd *et al.* (2016a).

Antioxidants measurements

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and lipid peroxidase activities were measured spectrophotometerically at 560 nm (SOD), 510 nm (CAT), 340 nm (GPx), 534 nm (LPx) and 540 nm NO using colorimetric kits (BIODIAGNOSTIC, Egypt) according to the following formulas:

SOD (U/ gm tissue) = [(% inhibition) × (3.75) × (1/ gm tissue)] × sample dilution.
CAT activity (U/g)=[(^{A} sample - ^{A} Standard)/ (^{A} Standard)]×[1/gm tissue used per test].
LPx activity (nmol/gm tissue) = [(^{A} sample) / (^{A} Standard)] × [10/gm tissue used].
GPx (U / g) = [(^{A} sample - ^{A} Standard)/ (^{A} Standard)] × [1/gm tissue used per test].
NO (µmol / L = [(^{A} sample) / (^{A} Standard)] × [50].

Gene expression

RNA extraction was performed according to the protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) and column DNase digestion was used to remove residual DNA. Primers used were supplied from Metabion (Germany) are listed in Table 1 and utilized in a 25-µL reaction and reverse transcription was performed in a Stratagene MX3005P real time PCR (Table 1).

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Table 1: Primers sequences, sizes of amplification and run conditions for RT-PCR.

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve (1 cycle)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation	
EF-1α	CCTTCAACGCTCAGGTCATC TGTGGGCAGTGTGGCAATC	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	62˚C 30 sec.	72˚C 30 sec.	94˚C 1 min.	62˚C 1 min.	94˚C 1 min.	Gröner *et al.* (2015)
IL-1β	GCTGGAGAGTGCTGTGGAA GAACATATAG									Castro *et al.* (2011)
HSP70	CCTGGAGCATCATGGCGTG CTCCTGTGTGGGGGTTTTCC TTTGGGCTTCCCTCCGTCTG				60˚C 30 sec.			60˚C 1 min.		Shi *et al.* (2015)
C5	GGACCCGGACCATACAACAG GGGGTTTTGCAGAGATGGGA									This study

The CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method (Yuan *et al.*, 2006) using the following formula:

ΔΔCt = ΔCt reference – ΔCt target, ΔCt target = Ct control – Ct treatment and ΔCt reference = Ct control- Ct treatment.

Statistical analysis

Analysis was performed using One-Way Analysis of Variance (ANOVA) and significant difference between groups based on the different concentrations of the dietary CUR supplement as main factor, was calculated using Duncan's multiple range tests by Statistical Package for the Social Sciences (SPSS) software (version 22.0). A value of $p<0.05$ was considered significant and data are expressed as means±standard error.

Results

Growth

No mortalities were recorded, except for the control and group fed with 1% CUR diet throughout the entire experiment. CUR diets markedly improved the growth parameters and group supplied with 1% CUR showed the highest increase ($p<0.05$), followed by 3 and 2% dietary CUR, respectively at 5 weeks while being challenged with cold pond water (Fig. 1 A, C and D). The most obvious ($p<0.05$) decrease in feed conversion ratio (FCR) was for 1% CUR group (Fig. 1B).

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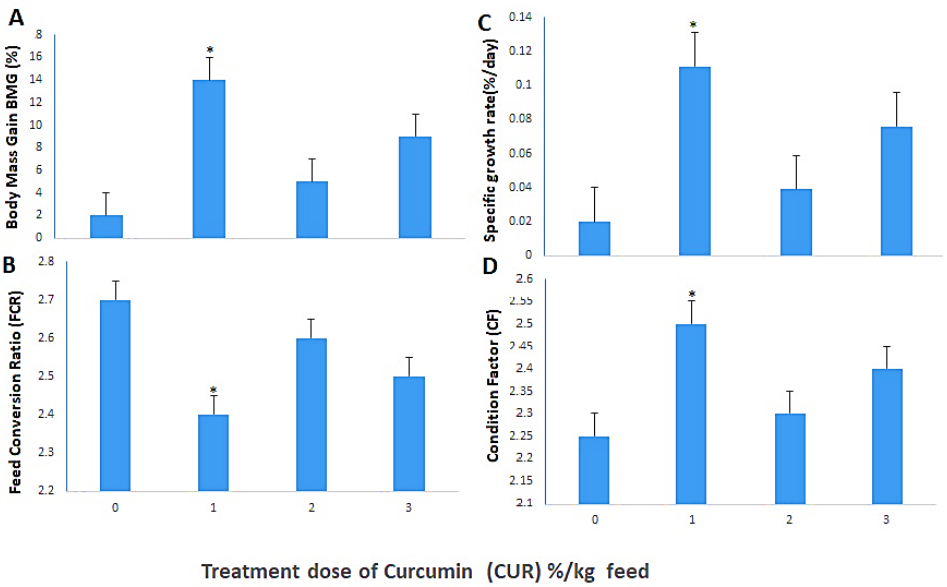

Figure 1: Growth parameters of Nile tilapia (*O. niloticus*) at 5 weeks after feeding with different levels of dietary CUR and exposure to cold stress. Means are mean (n=30)±SEM. Mean values with asterisk (*) are different significantly (p<0.05).

Antioxidant enzymes and nitric oxide (NO) activities

CUR supplementation showed significant increase in both SOD and CAT activities and 1% CUR diet showed the most significant (p<0.05) increasing compared with the control (Fig. 2A and B).

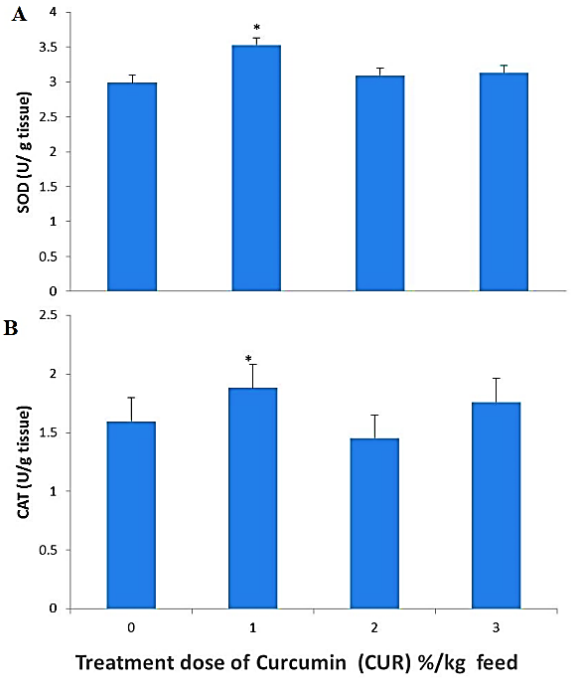

Figure 2: Effect of dietary CUR on SOD (A) and CAT (B) activities in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean (n=9)±SEM. Mean values with asterisk (*) are different significantly (p<0.05).

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GPx activities showed the highest (p<0.05) levels in 3 and 2% CUR incorporated diets (Fig. 3A and B). While, MDA levels revealed the most significant decrease (p<0.05) in 2% CUR group (Fig. 4)

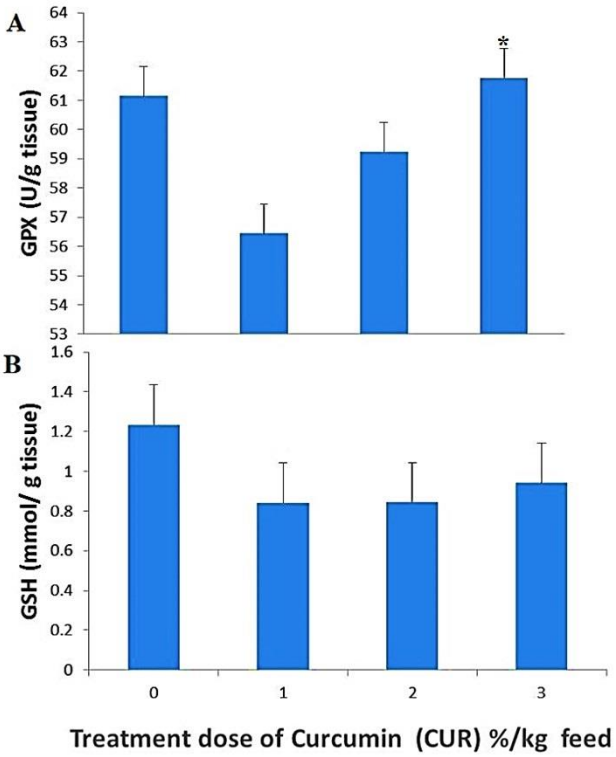

Figure 3: Effect of dietary CUR on GPx (A) and GSH (B) activities in liver of Nile tilapia (*O. niloticus* after exposure to cold stress. Values are mean (n=9)±SEM. Mean values with asterisk (*) are different significantly (p<0.05).

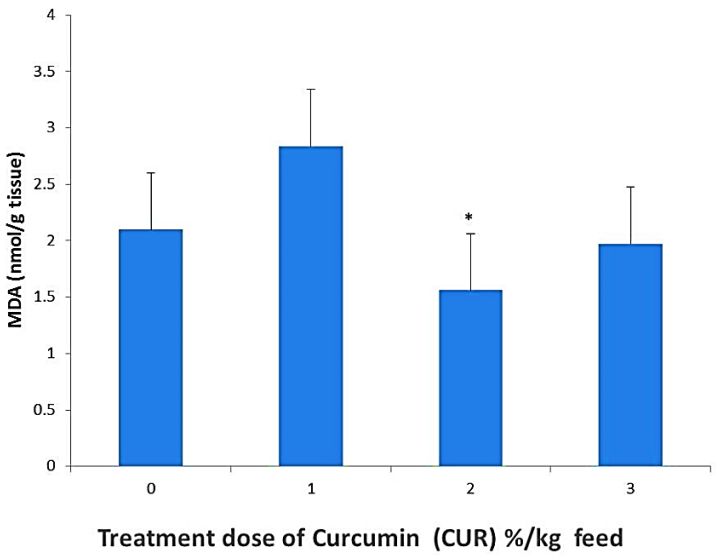

Figure 4: Effect of dietary CUR on MDA activity in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean (n=9)±SEM. Mean values with asterisk (*) are different significantly (p<0.05).

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The highest (p<0.05) NO level was recorded for 3% CUR group, followed by 1% group (Fig. 5).

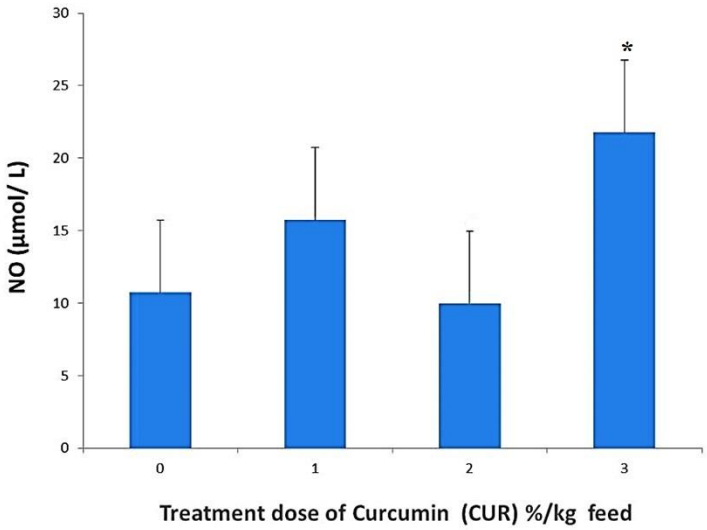

Figure 5: Effect of dietary CUR on NO level in liver of Nile tilapia *(O. niloticus*) after exposure to cold stress. Values are mean (n=9)± SEM. Mean values with asterisk (*) are different significantly (p<0.05).

Gene expression

Groups fed with 1% CUR incorporated diet showed the highest significant (p<0.05) upregulation of Heat Shock Protein 70 (HSP70), Interleukin-1β, (*IL-1*β) and Complement Component (C5) genes than the control group, followed by 2 and 3% groups, respectively (Fig. 6; Fig. 7A and B).

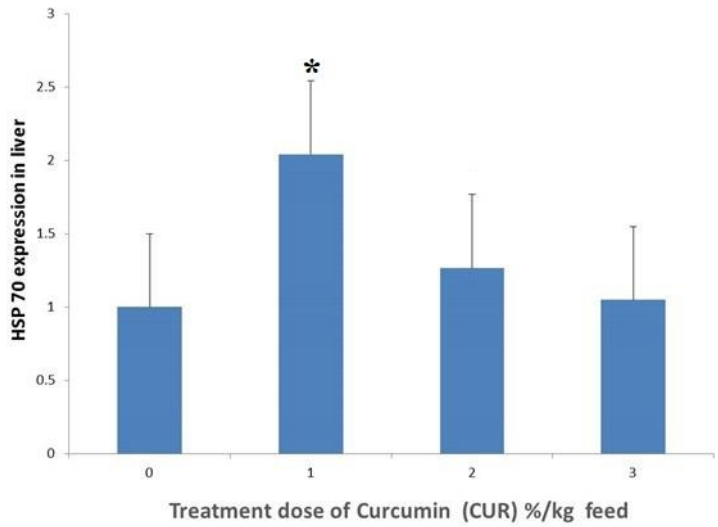

Figure 6: HSP70 gene expression in Nile tilapia (*O. niloticus*) after exposure to 6 weeks cold stress, Values are mean (n=9)±standard error. Mean values with asterisk (*) are different significantly (p<0.05).

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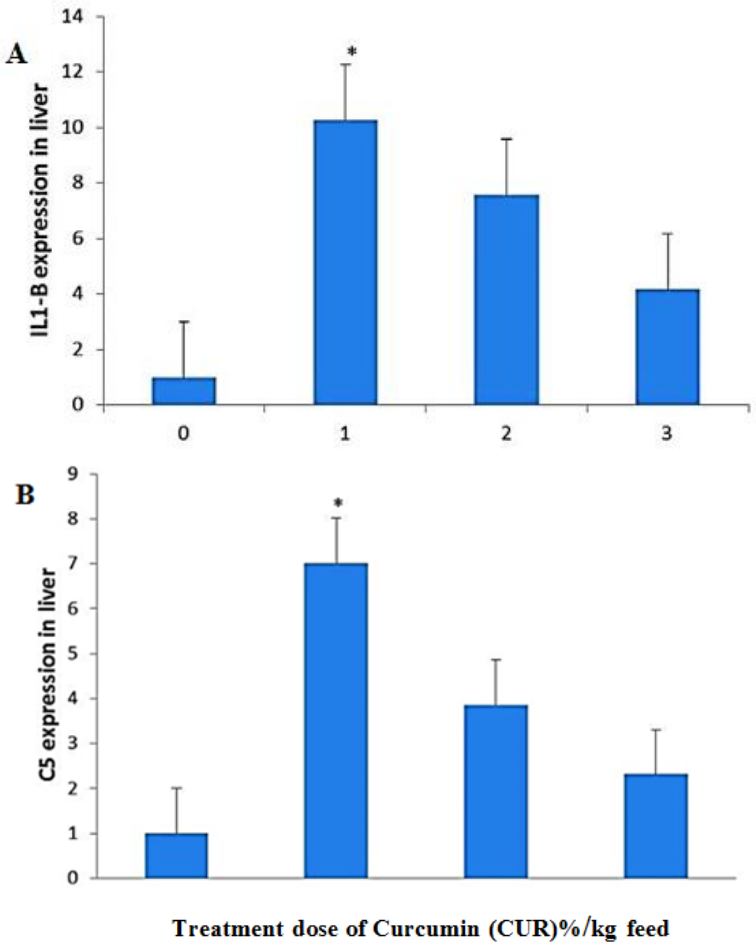

Figure 7: IL-1β (A) and C5 (B) genes expression in Nile tilapia (*O. niloticus*) after exposure to 6 weeks cold stress, Values are mean (n=9)±standard error. Mean values with asterisk (*) are different significantly (p<0.05).

Discussion

Many phytochemicals are considered as natural anti-oxidants that act through trapping free radicals and inhibiting the generation of oxygen anions (Chakraborty and Hancz, 2011). In the current study, we focused on CUR as a feed additive for Nile tilapia. Results showed that feeding dietary CUR significantly increased growth performance and anti-oxidative stress profiles throughout the entire experiment.

Growth parameters were significantly increased in all groups receiving CUR, with the most significant (p<0.05) increase for 1% CUR group. These findings are supported by those of Mahfouz (2015) as he found that adding CUR to *Oreochromis niloticus* diets showed a significant increase in growth parameters compared to control group. Also, Mahmoud *et al.* (2017) found that different concentrations of CUR markedly improved growth performance and feed utilization in the tilapia fish. Similarly, Enis Yonar *et al.* (2019)

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reported that the dietary CUR supplementation positively affected the growth of rainbow trout (*Oncorhynchus mykiss*). Those results may be attributed to digestive enhancing properties of CUR through improving lipase and trypsin activities in hepatopancreas and intestine; as well as amylase activity in hepatopancreas (Jiang, 2016; Enis Yonar *et al.*, 2019). In addition, enhancing nutrient utilization through boosting the activity of Na+/K+-ATPase and intestinal alkaline phosphatase, creatine kinase, and gamma-glutamyl transpeptidase can be mentioned, that are located in the intestinal brush border (Jiang, 2016).

Antioxidant systems are important to improve the imbalance in biological systems, that can be caused by different environmental stressors, among which changes in temperature plays a critical role (Parihar *et al.*, 1997; Madeira *et al.*, 2013). SOD, GPx, and CAT are important antioxidant enzymes (Livingstone, 2001; Somogyi *et al.*, 2007; Madeira *et al.*, 2013). Throughout the experiment, group fed with 1% CUR revealed the best significant (p<0.05) results for both SOD and CAT activities. Also, 3% CUR markedly (p<0.05) improved GPx and 2% CUR enhanced (p<0.05) the recorded MDA levels. Same findings were recorded in previous studies (Talpur, 2014; Elabd *et al.*, 2016b; Elabd *et al.*, 2017). In addition, Enis Yonar *et al.* (2019) reported that dietary CUR significantly improved tissue antioxidant capacity of rainbow trout. The significant increase in antioxidants enzymes activities may be attributed to the antioxidant and hepatoprotective activities effects of CUR (Boonla *et al.*, 2014; Mandal *et al.*, 2009; Kwak *et al.*, 2004) and ability to induce transcription of antioxidant enzyme through activation of signaling pathway for the nuclear factor erythroid 2 (Nrf2), which greatly participates in scavenging free radical, and thus obtain a great antioxidant properties (Kwak *et al.*, 2004); along with H-atom donation from phenolic group that is responsible for the well noticed antioxidant properties of CUR (Ak and Gülçin, 2008).

Nitric oxides play very important rules in the immune defense mechanism (Villamil *et al.*, 2002). In the present study, the most significant (p<0.05) increase in NO level was for 3% CUR group, followed by 1% group when compared with control group. Same results were recorded by Elgendy *et al.* (2016), who studied the effect of CUR on NO level and showed that different concentration of CUR significantly improved NO in Nile tilapia. This can be attributed to immunostimulating activities and ability of CUR to stimulate both the innate and humoral immune response in Nile tilapia (Boonla *et al.*, 2014; Mahmoud *et al.*, 2017).

On molecular level, Heat Shock Protein 70 (HSP70), Interleukin-1β, (*IL-1*β) and Complement Component (C5) genes expression were positively correlated with the current study findings, indicating that those genes can be used as molecular biomarkers for

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oxidative stress resulting from non-ambient cold temperatures in tilapia. Furthermore, immune related genes including *IL-1*β and C5 were enhanced by CUR incorporation, indicating their beneficial role. Jiang (2016) reported that CUR supplementation increased the relative mRNA expression of SOD, CAT and GPx in carp. These results came in agreement with Elabd *et al.* (2016a) and Elabd *et al.*, (2017). On the same instance, expression ratio of IL-8, IL-1β and TGF-β genes of the head kidney in rainbow trout fed with 1% and 2% lupin, mango and stinging showed up-regulation of target genes as compared to the control group (Awad *et al*., 2011). This can be attributed to hepatoprotective, immunostimulating and antioxidative activities of CUR in fish (Boonla *et al.*, 2014; Mandal *et al.*, 2009). Throughout the experimental period, there was a significant increase in growth performance and anti-oxidative stress profiles during exposure to natural challenging cold temperatures, suggesting the ability of dietary CUR to up-regulate the immune system during periods of increased risk, such as winter conditions.

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