

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-1 β* 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-1 β* 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 \pm 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,

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 into 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 $9.0 \pm 0.5^{\circ}\text{C}$. 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 spectrophotometrically 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:

$\text{SOD (U/ gm tissue)} = [(\% \text{ inhibition}) \times (3.75) \times (1/ \text{ gm tissue})] \times \text{sample dilution.}$

$\text{CAT activity (U/g)} = [(\text{A sample} - \text{A Standard}) / (\text{A Standard})] \times [1/\text{gm tissue used per test}].$

$\text{LPx activity (nmol/gm tissue)} = [(\text{A sample}) / (\text{A Standard})] \times [10/\text{gm tissue used}].$

$\text{GPx (U / g)} = [(\text{A sample} - \text{A Standard}) / (\text{A Standard})] \times [1/\text{gm tissue used per test}].$

$\text{NO } (\mu\text{mol / L}) = [(\text{A sample}) / (\text{A Standard})] \times [50].$

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).

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-1 α	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 <i>et al.</i> (2015)
IL-1 β	GCTGGAGAGTGCTGTGGAA GAACATATAG									Castro <i>et al.</i> (2011)
HSP70	CCTGGAGCATCATGGCGTG CTCCTGTGTGGGGGTTTCC TTGGGCTTCCCTCCGTCTG				60°C 30 sec.			60°C 1 min.		Shi <i>et al.</i> (2015)
C5	GGACCCGGACCATAACAACAG GGGGTTTTGCAGAGATGGGA									This study

The CT of each sample was compared with that of the positive control group according to the " $\Delta\Delta Ct$ " method (Yuan

et al., 2006) using the following formula:

$\Delta\Delta Ct = \Delta Ct_{reference} - \Delta Ct_{target}$, $\Delta Ct_{target} = Ct_{control} - Ct_{treatment}$ and $\Delta Ct_{reference} = Ct_{control} - Ct_{treatment}$.

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 \pm 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).

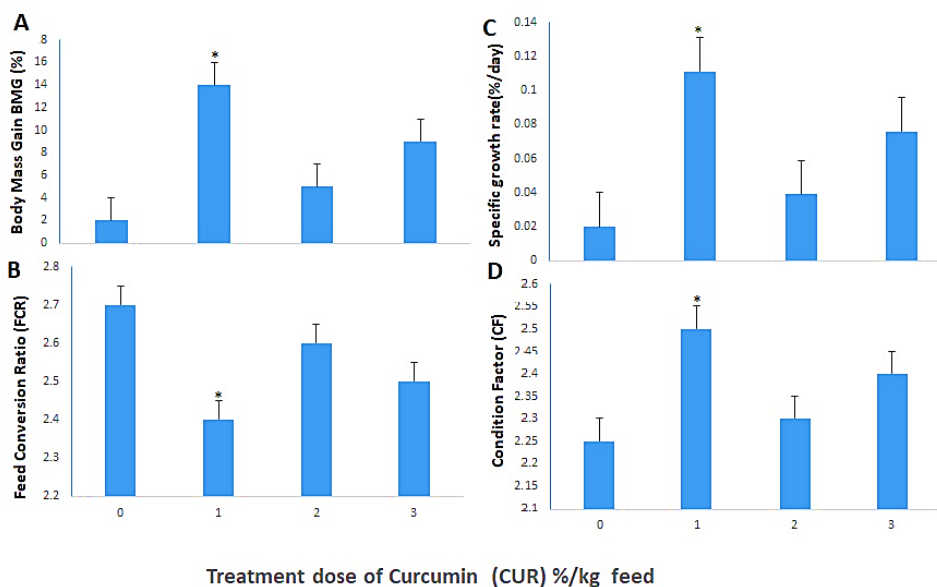


Figure 1: Growth parameters of Nile tilapia (*O. niloticus*) at 5 weeks after feeding with different levels of dietary CUR and exposure to cold stress. Means are mean ($n=30$) \pm SEM. Mean values with asterisk (*) are different significantly ($p < 0.05$).

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).

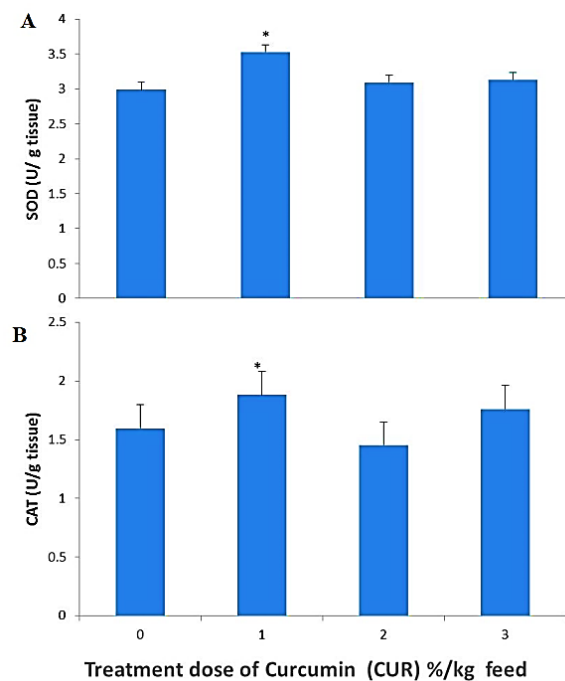


Figure 2: Effect of dietary CUR on SOD (A) and CAT (B) activities in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean ($n=9$) \pm SEM. Mean values with asterisk (*) are different significantly ($p < 0.05$).

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)

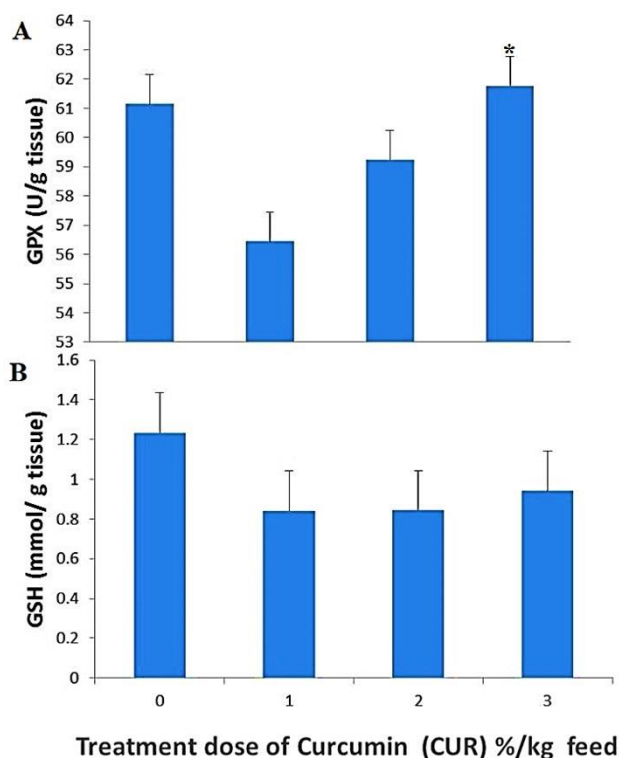


Figure 3: Effect of dietary CUR on GPx (A) and GSH (B) activities in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean ($n=9$) \pm SEM. Mean values with asterisk (*) are different significantly ($p<0.05$).

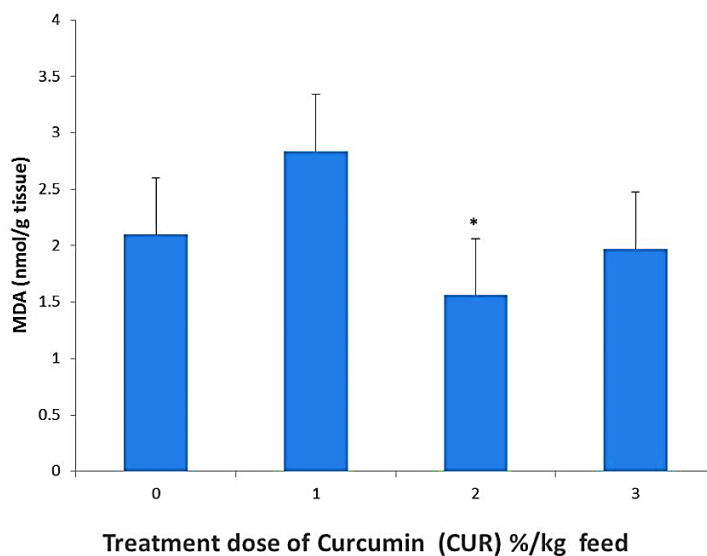


Figure 4: Effect of dietary CUR on MDA activity in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean ($n=9$) \pm SEM. Mean values with asterisk (*) are different significantly ($p<0.05$).

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

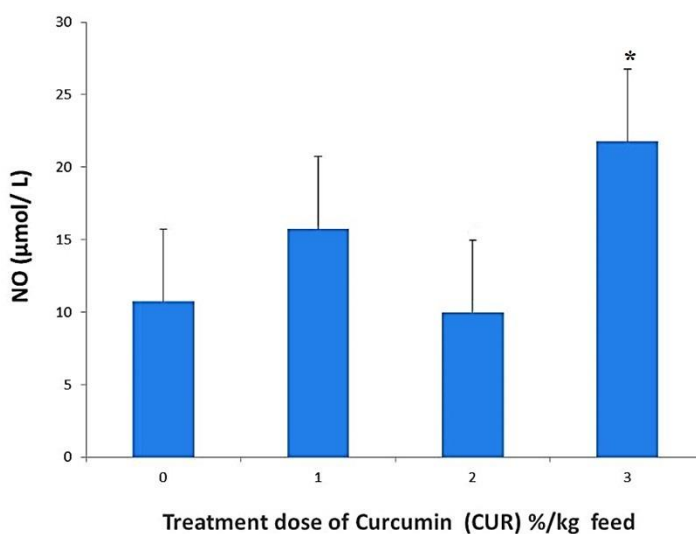


Figure 5: Effect of dietary CUR on NO level in liver of Nile tilapia (*O. niloticus*) after exposure to cold stress. Values are mean ($n=9$) \pm SEM. Mean values with asterisk (*) are different significantly ($p<0.05$).

Gene expression

Groups fed with 1% CUR incorporated diet showed the highest significant ($p<0.05$) upregulation of Heat Shock Protein 70 (HSP70), Interleukin-1 β ,

(*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).

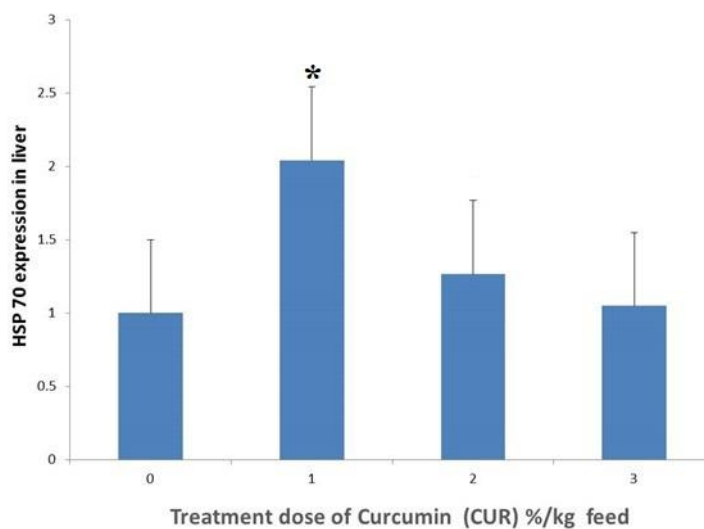


Figure 6: HSP70 gene expression in Nile tilapia (*O. niloticus*) after exposure to 6 weeks cold stress, Values are mean ($n=9$) \pm standard error. Mean values with asterisk (*) are different significantly ($p<0.05$).

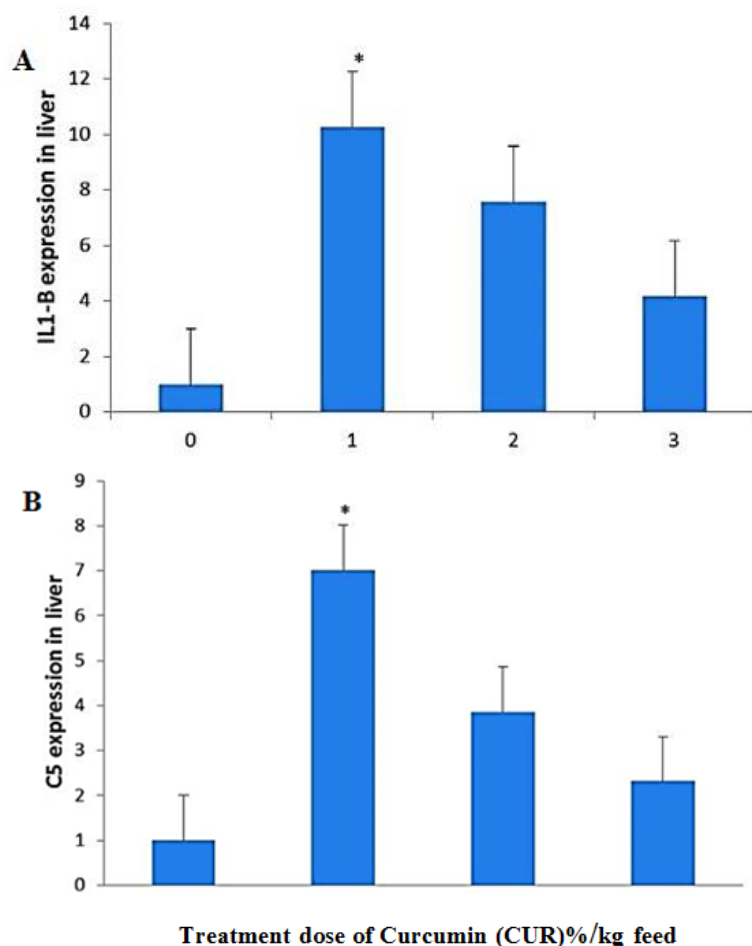


Figure 7: IL-1 β (A) and C5 (B) genes expression in Nile tilapia (*O. niloticus*) after exposure to 6 weeks cold stress, Values are mean (n=9) \pm standard error. Mean values with asterisk (*) are different significantly ($p < 0.05$).

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)

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-1 β , (*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

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-1 β 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.

References

- Ak, T. and Gülçin, I., 2008.** Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, 174(1), 27–37. DOI: 10.1016/j.cbi.2008.05.003.
- Atwood, H.L., Tomasso, J.R., Webb, K. and Gatlin, D.M., III 2003.** Low-temperature tolerance of Nile tilapia, *Oreochromis niloticus*: effects of environmental and dietary factors. *Aquaculture Research*, 34(3), 241–251. DOI: 10.1046/j.1365-2109.2003.00811.x.
- Awad, E., Mitchell, W.J. and Austin, B., 2011.** Effect of dietary supplements on cytokine gene expression in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 34(8), 629–634. DOI: 10.1111/j.1365-2761.2011.01271.x.
- Beere, H.M., Wolf, B.B., Cain, K., Mosser, D.D., Mahboubi, A., Kuwana, T., Taylor, P., Morimoto, R.I., Cohen, G.M. and Green, D.R., 2000.** Heat-shock protein 70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome. *Nature Cell Biology*, 2(8), 469–475. DOI: 10.1038/35019501.
- Bly, J.E. and Clem, L.W., 1992.** Temperature and teleost immune functions. *Fish and Shellfish Immunology*, 2(3), 159–171.
- Boonla, O., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan V, Pannangpetch P, Prachaney P, Greenwald SE. 2014.** Curcumin improves endothelial dysfunction and vascular remodeling in 2K-1C hypertensive rats by raising nitric oxide availability and reducing oxidative stress. *Nitric Oxide: Biology and Chemistry*, 42, pp. 44–53. DOI: 10.1016/j.niox.2014.09.001.
- Bowden, T.J., 2008.** Modulation of the immune system of fish by their environment. *Fish & Shellfish*

- Immunology*, 25(4), pp. 373–383. DOI: 10.1016/j.fsi.2008.03.017.
- Castro R, Zou J, Secombes CJ, Martin SA. 2011.** Cortisol modulates the induction of inflammatory gene expression in a rainbow trout macrophage cell line. *Fish & Shellfish Immunology*, 30(1), pp. 215–223. DOI: 10.1016/j.fsi.2010.10.010.
- Chakraborty, S.B. and Hancz, C., 2011.** Application of phytochemicals as immunostimulant, antipathogenic and antistress agents in finfish culture. *Reviews in Aquaculture*, 3(3), 103–119. DOI: 10.1111/j.1753-5131.2011.01048.x.
- Cossu, C., Doyotte, A., Jacquin, M.C., Babut, M., Exinger, A. and Vasseur, P., 1997.** Glutathione reductase, selenium-dependent glutathione peroxidase, glutathione levels, and lipid peroxidation in freshwater bivalves, *Unio tumidus*, as biomarkers of aquatic contamination in field studies. *Ecotoxicology and Environmental Safety*, 38(2), 122–131. DOI: 10.1006/eesa.1997.1582.
- Elabd, H., Wang, H.P., Shaheen, A., Yao, H. and Abbass, A.. 2016a.** Astragalus membranaceus (AM) enhances growth performance and antioxidant stress profiles in bluegill sunfish (*Lepomis macrochirus*). *Fish Physiology and Biochemistry*, 42(3), 955–966. DOI: 10.1007/s10695-015-0188-9.
- Elabd, H., Wang, H.P., Shaheen, A., Yao, H. and Abbass, A.. 2016b.** Feeding *Glycyrrhiza glabra* (liquorice) and Astragalus membranaceus (AM) alters innate immune and physiological responses in yellow perch (*Perca flavescens*). *Fish and Shellfish Immunology*, 54, 374–384. DOI: 10.1016/j.fsi.2016.04.024.
- Elabd, H., Wang, H.P., Shaheen, A., Yao, H. and Abbass, A.. 2017.** Anti-oxidative effects of some dietary supplements on Yellow perch (*Perca flavescens*) exposed to different physical stressors. *Aquaculture Reports*, 8, 21–30. DOI: 10.1016/j.aqrep.2017.09.002.
- Elgendy, M.Y., Hakim, A.S., Ibrahim, T.B., Soliman, W.S. and Ali, S.E., 2016.** Immunomodulatory effects of curcumin on Nile tilapia, *Oreochromis niloticus* and its antimicrobial properties against *Vibrio alginolyticus*. *Journal of Fisheries and Aquatic Science*, 11(3), 206–215. DOI: 10.3923/jfas.2016.206.215.
- El-Sayed, A.F.M. and Kawanna, M., 2008.** Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. *Aquaculture Research*, 39(6), 670–672. DOI: 10.1111/j.1365-2109.2008.01915.x.
- Gröner, F., Ziková, A. and Kloas, W., 2015.** Effects of the pharmaceuticals diclofenac and metoprolol on gene expression levels of enzymes of biotransformation, excretion pathways and estrogenicity in primary hepatocytes of Nile tilapia

- (*Oreochromis niloticus*). *Comparative biochemistry and physiology. Toxicology and pharmacology: CBP*, 167, 51–57. DOI: 10.1016/j.cbpc.2014.09.003.
- Jiang, J., 2016.** Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. *Aquaculture*. Translated by J. Jiang *et al.*, v. 463, 174–180. DOI: 10.1016/j.aquaculture.2016.05.040.
- Kleih, U., Linton, J., Marr, A., Mactaggart, M., Naziri, D. and Orchard, J., 2013.** Financial services for small and medium-scale aquaculture and fisheries producers. *Marine Policy*. (Social and cultural impacts of marine fisheries), 37, 106–114. DOI: 10.1016/j.marpol.2012.04.006.
- Kwak, M.K., Wakabayashi, N. and Kensler, T.W., 2004.** Chemoprevention through the Keap1-Nrf2 signaling pathway by phase 2 enzyme inducers. *Mutation Research*, 555(1–2), 133–148. DOI: 10.1016/j.mrfmmm.2004.06.041.
- Livingstone, D.R., 2001.** Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Marine Pollution Bulletin*, 42(8), 656–666. DOI: 10.1016/S0025-326X(01)00060-1.
- Lushchak, V.I. and Bagnyukova, T.V., 2006.** Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 143(1), 36–41. DOI: 10.1016/j.cbpc.2005.11.018.
- Lushchak, V.I., 2011.** Environmentally induced oxidative stress in aquatic animals. *Aquatic Toxicology (Amsterdam, Netherlands)*, 101(1), 13–30. DOI: 10.1016/j.aquatox.2010.10.006.
- Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C. and Diniz, M.S., 2013.** Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 166(2), 237–243. DOI: 10.1016/j.cbpa.2013.06.008.
- Mahfouz, M.E., 2015.** Ameliorative effect of curcumin on aflatoxin B1-induced changes in liver gene expression of *Oreochromis niloticus*. *Molecular Biology*, 49(2), 275–286. DOI: 10.1134/S0026893315020089.
- Mahmoud, H.K., A.Al-Sagheer, A., Reda, F.M., Mahgoub S.A. and Ayyat, M.S., 2017.** Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to *Aeromonas hydrophila* in *Oreochromis niloticus*. *Aquaculture*, 475, 16–23. DOI: 10.1016/j.aquaculture.2017.03.043.
- Mandal, M.N., Patlolla, J.M., Zheng, L., Agbaga, M.P., Tran, J.T., Wicker, L., Kasus-Jacobi, A., Elliott, M.H., Rao, C.V. and Anderson, R.E., 2009.** Curcumin protects retinal cells from light-and oxidant stress-induced cell death. *Free Radical Biology and Medicine*,

- 46(5), 672–679. DOI: 10.1016/j.freeradbiomed.2008.12.006.
- Parihar, M.S., Javeri, T., Hemnani, T., Dubey, A.K. and Prakash, P., 1997.** Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the freshwater catfish (*Heteropneustes fossilis*) to short-term elevated temperature. *Journal of Thermal Biology*, 22(2), 151–156. DOI: 10.1016/S0306-4565(97)00006-5.
- Shi, G.C., Dong, X.H., Chen, G., Tan, B.P., Yang, Q.H., Chi, S.Y. and Liu, H.Y., 2015.** Physiological responses and HSP70 mRNA expression of GIFT strain of Nile tilapia (*Oreochromis niloticus*) under cold stress. *Aquaculture Research*, 46(3), 658–668. DOI: 10.1111/are.12212.
- Somogyi, A., Rosta, K., Pusztai, P., Tulassay, Z. and Nagy, G., 2007.** Antioxidant measurements. *Physiological Measurement*, 28(4), R41-55. DOI: 10.1088/0967-3334/28/4/R01.
- Talpur, A.D., 2014.** *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. *Aquaculture*, 420–421, 71–78. DOI: 10.1016/j.aquaculture.2013.10.039.
- Villamil, L., Tafalla, C., Figueras, A. and Novoa, B., 2002.** Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). *Clinical and Diagnostic Laboratory Immunology*, 9(6), 1318–1323. DOI: 10.1128/CDLI.9.6.1318-1323.2002
- Yonar, E., Mişer Yonar, S., İspir, Ü. And Ural, M.Ş., 2019.** Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. *Achromogenes*. *Fish and Shellfish Immunology*, 89, 83-90. DOI: 10.1016/j.fsi.2019.03.038.
- Yuan, J.S., Chen, F. and Stewart, C., 2006.** Statistical analysis of real-time PCR data. *BMC Bioinformatics*, 7(1), 85. DOI: 10.1186/1471-2105-7-85.