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Dietary ginger improve growth performance, blood parameters, antioxidant capacity and gene expression in *Cyprinus carpio*

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Abstract

The present study evaluates the effect of dietary ginger (*Zingiber officinale*) on growth performance, haematological parameters, antioxidant status of common carp (*Cyprinus carpio*). Fish with initial weight of 14±1.95 g were allocated into twelve tanks at density of 18 fish per tank. Fish were fed four experimental diets containing 0 (as control diet), 0.5, 2 and 5% ginger powder for 56 days. After 56 days of culture, fish fed with 2 and 5% ginger diets showed significant increase in final weight, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) (p<0.05) compared to the control diet. Significant higher number of leucocytes (WBC), erythrocytes (RBC), haematocrit, lymphocyte, monocyte and neutrophil were found in the fish fed with 2 and 5% ginger diets compared to the control diet. Superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPX) enzyme activities and Malondialdehyde (MDA) levels were significantly higher in the groups fed 2 and 5% ginger diets (p<0.05). SOD, CAT and GPX mRNA expression levels of fish fed with 2 and 5% ginger diets were significantly down-regulated compared to the control diet (p<0.05). The results suggested that dietary ginger can improve the growth performance, health status and antioxidant capacity of common carp.

Keywords: Ginger, Antioxidant enzyme activity, Haematological indices, Reactive oxygen species, Growth performance.

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Introduction

Plants are natural sources of bioactive substances, which have useful effects in animal nutrition including appetite and feed intake induction, improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions (Lee *et al.,* 2014).

Improvement and protection of fish health in commercial production practices is an important factor in aquaculture industry. Medicinal plants are very useful in aquaculture because they have natural organic components that help reducing the mortality of fishes because of various pharmacological actions. In addition, they do not have any adverse effect to the environment and organism's health (Vahedi *et al.,* 2015). Today, aquaculture products have gained a special place among other human food sources. One of the basic practices in order to supply part of human need to protein is fish culture. Therefore, due to increasing human population in recent decades, the researchers try to present the new ways for increasing the production in the shortest possible time, along with the least side effects to the cultured organisms. This subject has led the researchers to focus more on using, additives and biological diets, such as enzymes, probiotics, proteins, and medicinal plants.

In many studies, the effects of different parts of plant species (flowers, leaves, seeds and roots) had been reported to influence in different ways such as growth improvement, antioxidant status, none specific immune response and increasing the survival rate in some commercial fish species such as African catfish, *Clarias gariepinus* (Dada and Ikuerowo, 2009; Soosean *et al*., 2010), tilapia *Orechromis mossambicus* (Immanuel *et al*., 2009) and common carp, *Cyprinus carpio* (Alishahi *et al.,* 2010; Pakravan *et al.,* 2012; Fallahpour *et al.,* 2014), and Major carp, *Catla catla* (Arulvasu *et al*., 2013).

Ginger (*Zingiber officinale*) is a medicinal plant that is widely used in traditional medicine and available as dietary supplement in medical sciences. From ancient times due to diversity of the active compounds in ginger it had been used in treatment of various diseases ginger generally contains alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fiber, carbohydrate, vitamins, carotenoids and minerals (Otunola, 2010; Prakash, 2010); and various pharmacological properties of ginger such as antioxidant, anti-tumor, anti-apoptosis, anti-inflammation, anti-hyperglycemia, anti-cough and anti-colds have been known and reported (Dadfar *et al*., 2014).

Common carp that belongs to the family Cyprinidae, is one of the most important species due to its rapid growth rate, high economic profit and ease of cultivation that can be seen in most natural environments all over the world (Tokur *et al*., 2006).

Some studies have reported that dietary ginger could enhance the growth performance, survival rate, non-specific immune response, physiological status, resistance to bacterial diseases and pH stress (Chang

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et al., 2012; Arulvasu *et al.,* 2013; Chan *et al.,* 2014; Levy *et al.,* 2015; Nan *et al.,* 2015).

Previous studies have shown that the antioxidant defense system, including antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and some functional molecules (such as: albumin, ceruloplasmin, ascorbic acid, etc), is developed in animals in order to stabilize the minimum levels of ROS in the cell (García-Triana *et al.,* 2010; Sheikhzadeh *et al.,* 2012; Yuan *et al.,* 2015; Zhang *et al.,* 2013).

Generally, environmental changes (such as seasonal and daily temperature fluctuations) or anthropogenic factors can make hypoxic status (Zhang *et al.,* 2013). The hypoxia subjection would cause to increase the production and the release of reactive oxygen species (ROS) and oxidative stress (Jusman *et al.,* 2010). ROS are well known to be responsible for lipid peroxidation, protein degradation, DNA damage, and apoptosis in vertebrates. In addition, these effects can permanently lead to the break of free radicals in the destructive process of lipid peroxidase (Ahmad *et al.,* 2000). The oxidative stress happen when a misbalance occurs between the production of ROS and antioxidant systems in the body of organism that can be quantified by measurement of the antioxidant enzyme activity and antioxidant gene expression procedures (Trenzado *et al.,* 2006).

The aim of this study was to further improve our knowledge of the antioxidant mechanism of dietary ginger on the growth parameters, survival and antioxidant enzymes activities, CAT, SOD, GPX mRNA levels and some hematological parameters of cultured Common carp (*Cyprinus carpio*) Juveniles.

Materials and methods

Fish, experimental design and preparation of ginger feed

Common carp juveniles with mean weight of 14±1.95 g (mean±SD) were collected from the Zahak Fish Reproduction and Rearing Co. Ltd, Zabol, Iran. The fishes were fed with the basal diet for 2 weeks for adaptation to the experimental conditions and then were divided into four treatment groups and three replication (18 fishes in tanks with 75 L capacity). Fishes were fed with various concentrations of ginger (supplied by Golha corporation, Iran) powder (0 “control”, 0.5, 2 and 5 % of diet) for 56 days (Table 1). The daily feeding rate was 3% of body weight in three times (08:00, 14:00 and 18:00). During the experimental period, water temperature, dissolved oxygen and pH were measured daily and maintained at 19.8±1.5°C, 6.2±0.6 mg L^{-1}, and 8.1±0.2, respectively. Continuous aeration was provided in each tank through an air stone connected to a central air compressor. In order to prepare the experimental diets, all ingredients were thoroughly mixed with various levels of ginger powder, and water was added to produce pellets of approximate 2 mm in diameter (Cerezuela, 2008). The pelleted diets were dried, sieved and stored at -20 °C until used.

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In order to evaluate the bioenergetics parameters at the end of experimental period, Weight Gain (WG%), Specific Growth Rate (SGR %/day), Feed Conversion Ratio (FCR) and Survival Rate (%) were calculated according to the following equations (Mahghani *et al.*, 2014): $WG(\%)=(Wt-W_0)\times100/W_0$, $SGR=(\ln Wt-\ln W_0)\times100/t$, FCR=dry feed fed/ Wet weight gain, Survival rate $=(Nt/N_0)\times100$. Here Wt and W_0 are final and initial body weights (g), respectively, t is duration of experimental days, N_0 is the initial number of fishes and Nt is the final number of fishes.

Table 1: Formulation and proximate composition of experimental diets (%).

Ingredients	Experimental diet			
	0	**0.5%**	**2%**	**5%**
Fish meal	37.5	37.5	37.5	37.5
Soybean meal	16.5	16.5	16.5	16.5
Wheat	20	20	20	20
Canola oil	6.5	6.5	6.5	6.5
Corn	5	5	5	5
DCP	2.05	2.05	2.05	2.05
Carboxy methyl mellulose	2	2	2	2
$CaCO_3$	1.89	1.89	1.89	1.89
Vitamin permix[1]	0.4	0.4	0.4	0.4
Mineral premix[2]	0.25	0.25	0.25	0.25
Vitamin C	0.08	0.08	0.08	0.08
Colin	0.15	0.15	0.15	0.15
Filler	5	4.5	3	0
ginger powder	0	0.5	2	5
Proximate composition (% DM[3])				
Protein	32	32.5	31.9	32.3
Fat	13.4	12.5	12.8	13.1
Moisture	4.8	5	4.9	5.1
Ash	14.5	14.6	14.4	14.2

[1]Provided per kilogram of feed: vitamin D, 1.28 mg; vitamin E, 136 mg; thiamine, 8 mg; riboflavin, 16 mg; 3 pyrodoxine-Cl,16 mg; vitamin C, 80 mg; calcium pantothenate, 17.4 mg; biotin, 0.16 mg; folic acid, 4.8 mg; niacin, 40 mg; vitamin B,0.016 mg; menadion bisulphite, 20 mg

[2]Provided per kilogram of feed: magnesium, 625 mg; potassium, 509 mg; calcium, 465 mg; zinc, 100 mg; iron, 62 mg; manganese, 13 mg; copper, 6 mg

[3]DM=dry matter

Haematological and biochemical parameters

At the end of trial, the hematological parameters were measured by taking 2 mL blood samples from the caudal vein of three fishes from each tank. The blood samples were then suspended in heparinized tubes, and then values of red blood cell (RBC), white blood cell (WBC), hematocrit (PCV), Neutrophil, Lymphocyte and Monocytes were measured (Faggio *et al.*, 2014).

For biochemical analysis, blood samples of fishes were taken from the caudal vessels by using heparinized syringes. Prior to sampling, the fish were anesthetized with 200 mg L^{-1} MS 222 (Faggio *et al.*, 2013). Blood sera were separated by centrifuging the sample at 3,000 rpm for 10 min

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(Heraeus Labofuge 400) and the sera were removed with a disposable transfer pipette (Akrami *et al.,* 2015). Then, the SOD (Superoxide dismutase) activity was determined based on the method previously described by Beyer and Fridovich (1987). The SOD activity was reported in units of enzyme/gram hemoglobin. One unit of SOD corresponds to the enzyme concentration required to inhibit the chromogen produced nitro blue tetrazolium by 50% in 1 min under standard conditions. GPX (Glutathione peroxidase) activity was measured according to the method explained by the previously described procedure (Paglia and Valentine, 1967). GPX activity was expressed in milliunits/mg hemoglobin. One specific unit is defined as 1 mmol of nicotinamide adenine dinucleotide phosphate (NADPH) needed with 1 g of hemoglobin to be converted to NADPH in 1 min of reaction. CAT (Catalase) activity was measured according to the method proposed previously (Aebi, 1984). The CAT activity is defined in specific units/mg hemoglobin. One unit of CAT corresponds to the amount of enzyme needed to decompose H_2O_2 in phosphate buffer, at pH 7.0, in 1 sec of reaction. Malondialdehyde (MDA) was measured by using a competitive enzyme-linked immunosorbent assay kit (Zellbio Co., GmbH, Germany) according to the manufacturer's instructions as previously described by Zhang *et al.* (2013).

Expression of CAT, SOD and GPX mRNAs

In order to compare the expression of mRNA levels, individual liver tissue from each treatment group (n=6) were randomly collected at the end of trial and frozen and kept at -80°C until use. Total RNA extractions from liver samples were carried out by using Takapou Zist Kit (Tehran, Iran) following the manufacturer's instruction. RNA integrity was verified by ethidium bromide staining of 28S and 18S ribosomal RNA bands on 1.2% agarose gel. To remove DNA contaminants, the extracted RNA was treated with RNA-Free DNase (Takara, Japan) and reverse transcribed to cDNA by a Superscript cDNA synthesis kit (AccuPawer® CycleScript RT PreMix, Germany) following the manufacturer's instructions.

The mRNA expression levels of CAT, SOD and GPX genes in the liver of common carp were evaluated by fluorescent real-time quantitative PCR. The specific primers for CAT, SOD, GPX and β-actin (housekeeping gene) were designed according to the cDNA sequences of common carp in Gen Bank (Table 2) (Jiang *et al*., 2016), and thermocycling conditions as indicated in Table 2. All primers were synthesized by Takapou Zist Co., Ltd. and amplified fragment length of 75–295bp. Real-time quantitative PCR was conducted in a quantitative thermal cycler (Mastercycler® eprealplex; Eppendorf, Germany). Each reaction was performed in a total volume of 20 μL, containing 0.5 μL of each primer (15 mM), 2 μL of the diluted first strand

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cDNA product, 10 µL of Green qPCR Master Mix (Yekata Tajhiz Azma Co., Iran) and 7 µL of sterilized double-distilled water. Three technical replicates for each sample were performed and the threshold cycle (CT) was determined manually for each run. PCR efficiencies for each set of primers were determined using serial 10-fold dilutions of cDNA and resulting plots of CT vs. the logarithmic cDNA dilution, using the efficiency equation (E):

$$E = 10^{(-1/slope)}$$

Gene expression data were analyzed using the $2^{-\Delta\Delta CT}$ method after verification that the primers amplified with an efficiency of from 97 to 99% (Gharaei *et al*., 2011), and data for all treatment groups were compared to the control group.

Table 2: Real-time PCR primer sequences and thermocycling condition.

Target genes	Primer	Primer sequence (5´— 3´)	Thermocycling condition	Accession no.	Product length (bp)
SOD	F	TGGCGAAGAAGGCTGTTTGT	95 ◦C 30 s, 35 cycles of 95 ◦C 5 s, 60.4◦C 30 s and 72 ◦C 30 s	JF342355	85
	R	TTCACTGGACCCGTCT			
CAT	F	CTGGAAGTGGAATCCGTTTG	95 ◦C 30 s, 35 cycles of 95 ◦C 5 s, 54.5◦C 30 s and 72 ◦C 30 s	JF411604	75
	R	CGACCTCAGCGAAATAGTTG			
GPX	F	CCTTCCCATCCCACCAGTTT	95 ◦C 30 s, 35 cycles of 95 ◦C 5 s, 60 ◦C 30 s and 72 ◦C 30 s	FJ656211	118
	R	TGCGGAGTCACCGTTCACAT			
β-Actin	F	CGTGATGGACTCTGGTGATG	95 ◦C 30 s, 35 cycles of 95 ◦C 5 s, 60 ◦C 30 s and 72 ◦C 30 s	M24113	295
	R	TCGGCTGTGGTGGTGAAG			

Statistical analysis

Data were tested for normality and homogeneity of variance before application of parametric tests. The data related to growth performance indices, Hematological indices, the liver mRNA gene expression levels were subjected to one-way analysis of variance (ANOVA) and if significant differences (p<0.05) were found, Tukey's post hoc test was used for ranking the groups by using of the SPSS (version 18).

Results

Growth performance

The effects of ginger powder in common carp diet on the growth performance and survival rate are represented in Table 3. The results of statistical analysis of growth performance indices showed significant improvement (p<0.05) in final weight, weight gain, SGR and FCR in fishes fed 2 and 5% ginger diets compared with the control diet at the end of experiment. No mortality was recorded during feeding trial and survival rate was 100% in all treatment groups.

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Table 3: Growth parameters and survival rate of common carp juveniles fed with different ginger level diets for 56 days feeding trial (data expressed as Mean±SE). Means marked by different letters are significantly different (p<0.05).

Performance indices	Dietary groups			
	control	0.5 %	2%	5%
Initial weight (mg)	14.51± 0.30	14.87± 0.49	13.88± 0.63	13.90± 0.66
Final weight (mg)	42.51± 3.46^{a}	45.67± 2.56^{a}	60.08± 3.10^{b}	71.20± 4.31^{c}
Weight gain (%)	19.3±2.9^{a}	20.7±3.3^{a}	33.3 ±4.12^{b}	41.2± 5.74^{c}
SGR (% day^{-1})	1.92± 0.13^{a}	2.00± 0.17^{a}	2.6± 0.25^{b}	2.9± 0.22^{c}
FCR	1.79± 0.05^{a}	1.75± 0.07^{a}	1.55± 0.09^{b}	1.42± 0.04^{b}
Survival rate (%)	100	100	100	100

Haematological and biochemical parameters

The effects of different levels of dietary ginger on the haematological parameters are presented in Table 4. WBC, RBC, haematocrit, lymphocyte, monocyte and neutrophil levels were significantly higher in the fish fed with 2 and 5% ginger powder diets, compared with the other groups (p<0.05).

Table 4: Hematological parameters of common carp juveniles fed with different ginger level diets for 56 days feeding trial (data expressed as M ± SE). Means marked by different letters are significantly different (p<0.05).

Parameters	Dietary groups			
	Control	0.5 %	2 %	5 %
WBC (10^4 cell mm^{-3})	24.83±3.1^{a}	32.41 ±2.2^{b}	47.43±3.1^{c}	49.33±3.3^{c}
RBC (10^6cell mm^{-3})	1.6±0.3^{a}	1.5±0.2^{a}	1.8±0.15^{b}	1.9 ±0.25^{b}
Haematocrit(%)	30.12±2.23^{a}	31.4±3.1^{a}	38.2±2.5^{b}	41.4±3.8^{b}
Lymphocytes(%)	32.00±1.7^{a}	35.00±1.6^{a}	42.30±2.3^{b}	47.50±2.60^{b}
Monocytes(%)	19.66±0.2^{a}	20.5±0.17^{a}	26.7±0.2^{b}	28.5±0.3^{b}
Neutrophils (%)	28.33±0.71^{a}	30.53±0.55^{a}	35.60±0.66^{b}	36.36±0.95^{b}

The Antioxidant enzyme activity and malondialdehyde levels were presented in Table 5. SOD, CAT and GPX enzyme activities and MDA level of the fish fed 2 and 5% ginger-supplemented diets were significantly higher than that of fish fed with the control and 0.5% ginger-supplemented diets (p<0.05).

Table 5: Antioxidant capacity parameters of common carp juveniles fed with different ginger level diets for 56 days feeding trial (data expressed as Mean± SE). Means marked by different letters are significantly different (p<0.05).

Parameter	Diet group			
	control	0.5%	2%	5%
SOD(unit ml^{-1})	46.28±0.62^{a}	42.42±1.29^{ab}	34.92±1.33^{b}	31.65±3.98^{b}
CAT(unit ml^{-1})	3.98±0.70^{s}	3.71±0.13^{s}	2.20±0.39^{b}	2.32±0.33^{b}
GPX (unit ml^{-1})	30.68±7.20^{a}	28.60±4.50^{ab}	23.50±3.25^{b}	20.91±3.1^{b}
MDA (nmol ng^{-1})	5.8±0.52^{a}	5.5±0.48^{a}	4.3±0.26^{b}	3.9±0.3^{b}

Expression of CAT, SOD and GPX mRNAs

The mRNA levels of common carp liver genes expression relative to β-Actin are showed in Table 6. The expression of SOD, CAT and GPX genes in fish liver was affected by the ginger supplementation. SOD and CAT

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mRNAs expression levels had significant differences (p<0.05) between fishes fed with 2 and 5% ginger-supplemented diets and fish fed in other treatment groups. GPX gene expression levels showed significant increase (p<0.05) in fishes received 5% ginger-supplemented diet in comparison to the other treatment groups.

Table 6: Relative mRNA levels of common carp juvenile's liver exposed to four dietary ginger powder levels for 56 days feeding trial (data expressed as Mean±SE and n=5). Means marked by different letters are significantly different (p<0.05).

Genes	Diet group			
	control	0.5%	2%	5%
SOD	6.90±0.51[a]	5.79±0.11[a]	3.53±0.24[b]	3.06±0.20[b]
CAT	9.79±0.68[a]	8.12±0.22[a]	5.18±0.32[b]	3.20±0.49[c]
GPX	8.24±0.31[a]	8.17±0.41[a]	6.76±0.42[ab]	4.15±0.18[b]

Discussion

Among the phytochemical agents application in aquaculture, ginger as a dietary supplementation has been widely used in recent decade. The present study indicated that dietary ginger levels of 2 and 5% could improve the final weight, weight gain, SGR and FCR of common carp. The previous studies demonstrated that administration of ethanolic extract and powdered ginger increases the growth performance in *Oncorhynchus mykiss* (Shaluei *et al*., 2016), *Lates calcarifer* (Talpur *et al.,* 2013), *Macrobrachium rosenbergii* (El-Desouky *et al.,* 2012) and *O. mykiss* (Nya and Austin, 2009). However, in our study the higher growth was dependent to the ginger level, suggesting that 2 and 5% ginger diets were most favorable for growth performance of common carp. So, improvement of FCR could be due to the role of ginger as an appetizer. Thus, the increase of the growth rate could be due to enhancement of digestibility and metabolic process. Common carp as an omnivorous fish has a long intestine, in order to have a better absorption capacity related to antiflammatory and antiproliferation effects (Pérez-Sánchez *et al.,* 2015). In addition, the functional mechanisms of dietary ginger effect on the growth of fishes are still not well understood and need to be further studied.

Generally, haematological and biochemical parameters are indicators of fish health condition as well as the physiological status of an organism (Gharaei *et al.,* 2016). White blood cells are identified as indicators of health status in fish and play an important role in regulation of immunologic function (Ballarin *et al.,* 2004; Van Hai, 2015), because their numbers enhance immediately when infections arise.

In this study, the WBC and RBC counts, haematocrite, lymphocyte and other blood cells increased in the 2 and 5% ginger diets which demonstrate the immunostimulatory and anti-infection properties of ginger. Improvement of blood cell counts were also reported by Talpur *et al.* (2013) and Nya and Austin (2009) who obtained enhanced blood cells in *L. calcarifer* and *O.*

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mykiss after feeding them with ginger. It could also be related to bioactive compounds of ginger including polyphenols, flavonoids, tannins and saponins that play anti-infection role in fish. Moreover, the increase of RBC count in fish fed with 2 and 5% ginger diets implies the suitable health effect in them due to bioactive components in ginger. Similar results have been reported about the ginger effect on RBC count in *L. calcarifer* (Talpur *et al.,* 2013) and *O. mykiss* (Nya and Austin 2009). So, many studies demonstrated the effect of immunostimulant herbal plants on blood cells because they induce immune system in aquatic animals (Sahu *et al.,* 2007; Harikrishnan *et al.,* 2011; Talpur and Ikhwanuddin., 2012; Hwang *et al.,* 2013; Van Hai, 2015).

Similar to other blood cells, lymphocytes, monocytes and neutrophils levels were improved by 2 and 5% ginger diets. These results could be implied to the task of these cells, because they increase when the organism is exposed to infections caused by pathogens and other foreign toxic material. These finding are comparable with Talpur *et al.* (2013), Talpur and Ikhwanuddin (2012) and Nya and Austin (2009).

The haematocrite (Hct) % increase in 2 and 5% ginger fed groups which could be explained by immunostimulatory effect of ginger. Typically, a higher Hct level signifies the blood sample's ability to transport oxygen and also serve as an indicator of health conditions (Birchard, 1997). Similarly, increased Hct % was observed in *L. calcarifer* and *O. mykiss* fed by ginger diet in Talpur *et al.,* (2013) and Nya and Austin (2009) studies.

The earlier studies stated that more than 60 different medicinal plant species were used for the improvement of fish health and disease management in aquaculture (Van Hai, 2015) and some of them could enhance the antioxidant enzyme activities in organisms for adaptation to the environmental stress which may lead to excessive ROS production (Livingston, 2001; Lukaszewicz-Hussain, 2010). SOD, CAT and GPX enzymes involving in antioxidant system are identified as the first defense mechanism against oxidative stress by radical process and phagocytosis within damaged tissue (Abhijith *et al.,* 2016). However, the major role of these enzymes is to neutralize the ROS produced in different organs (Zhang *et al.*, 2011). The significant decrease which was observed in SOD, CAT and GPX enzyme activities in liver of fishes fed with 2 and 5% ginger diets suggested that the ginger stronger O_2 quenching activity than these enzymes, and dietary ginger could relieve the oxidative stress and maintain the healthy condition of individuals. Our finding corroborates the study by Zhang *et al.,* (2011) who reported significant decreases in SOD and CAT expression levels following the administration of astaxanthin in *L. vannamei*.

Generally, the production of reactive aldehyde is used as a biomarker to

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measure the level of oxidative stress in an organism (Del Rio *et al.,* 2005). Besides, ROS degrade polyunsaturated lipids by forming malondialdehyde (MDA) (Zheng *et al*., 2012). The decline of MDA level in fish fed with 2 and 5% ginger diets indicated that ginger could prevent the lipid damage caused by free radicals (Talas and Duran, 2012). The results may be explained that some of the existing compounds in ginger such as flavonoids could reduce the oxidative stress by ROS scavenging (Fuat Gulhan *et al.,* 2012).

Many studies reported that the ingredients or nutritional factors in feed could affect the expression of antioxidant enzymes of fish (Lin *et al.,* 2007; Tovar-Ramírez *et al.,* 2010; Zheng *et al.,* 2012).

But the effects of dietary ginger on antioxidant enzymes mRNA levels in fish are not consistent in previous studies. In present study, the expression levels of CAT, SOD and GPX mRNAs were examined among fishes fed with all treatment diets. The lower expression levels of these genes recorded in fishes fed with 2 and 5% ginger diets and their expression profiles were similar together and parallel with antioxidant enzymes activity levels (Table 5). Also, it was demonstrated that increase in the expression level of these genes was attributed to increase in expression level of Nfr2 gene and decrease in expression level of Keap1 (Sukumaran *et al.,* 2016). Because the Nfr2 gene plays as a key moderator in primarily regulating the expression of antioxidant enzymes and have been shown to increase the expression levels of SOD and GPX associated with increasing of the Nfr2 gene expression (Chen *et al.,* 2015). The results of this study suggested that dietary ginger could partially alleviate oxidative stress by involvement in the expression of Nfr2 gene in common carp, which could help to enhance the scavenging ability of ROS in tissue cells.

In conclusion, the present study revealed that dietary ginger improves growth performance and feed utilization efficiency of common carp. In addition, the supplementation of ginger in fish feed can enhance the antioxidant capacity in response to oxidative stress in enzymatic and molecular functional levels in the tissue cells, which results in the increase of stress resistance in fish.

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