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Coffee bean in common carp, *Cyprinus carpio* L. diets: Effect on growth performance, biochemical status, and resistance to waterborne zinc toxicity

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ABSTRACT

The present study was undertaken to evaluate the use of roasted coffee powder (RCP; *Coffea arabica*) in practical diets for common carp, *Cyprinus carpio* L. to improve their growth, biochemical status, and resistance against Zn toxicity. However, RCP was added to the ingredients of tested diets to represent 0.0 (control), 0.50, 1.0, 2.0, or 5.0 g/kg diet. Fish (10.2 ± 0.42 g) were distributed into various treatments at a rate of 20 fish per 100-L aquarium and fed one of the experimental diets for 10 weeks in triplicates. After the feeding trial, fish from each treatment were further-exposed to 5.0 mg Zn/L for 7 days. It is noticed that final fish performance was not significantly ($P < 0.05$) affected by increasing RCP levels up to 1.0 g/kg after which fish growth declined. Moreover, fish fed diets containing 2.0–5.0 g RCP/kg consumed less diet than the other treatments giving highest FCRs (1.46 and 1.53, respectively), whereas fish fed 0.0–1.0 RCP/kg diet consumed approximately the same feed amount giving the same FCR (1.30–1.33). Furthermore, energy utilization decreased significantly at 2.0–5.0 g RCP/kg. No significant differences were observed in fish survival and its range was 93.3–96.7% among the different treatments. The supplementation of RCP reduced significantly protein and lipid contents and improved significantly ash content in whole-fish body. Furthermore, RCP inclusion resulted in significant decreases in plasmatic glucose, protein, and lipids, whereas their highest values were obtained with fish fed the control diet. Contrarily, plasmatic AST, ALT, creatinine, and uric acid values increased significantly and nitroblue tetrazolium (NBT) was significantly higher at RCP levels over 1.0 g/kg diet. After Zn exposure, Zn effect was more severe in fish fed RCP-free diet than those fed RCP-enrich diets. In control Zn-exposed fish, plasmatic glucose, total protein, and total lipids were significantly higher; meanwhile, plasmatic AST, ALT, creatinine, and uric acid levels were lower than those in fish fed RCP levels. In addition, NBT decreased due to Zn exposure. Likewise, Zn residues in whole-fish body decreased significantly with increasing RCP levels in diets and lowest daily Zn content was detected in fish fed 2.0–5.0 g RCP/kg diet. These results suggested that RCP supplementation cannot improve fish growth and feed utilization but it could improve their immunity and reduce the impact of water-born Zn toxicity and bioaccumulation in fish body.

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1. Introduction

Nowadays medicinal herbs are used as immuno-stimulants for human all over the world (Harikrishnan et al., 2011). The medicinal plants are rich in a wide variety of nutrients and antioxidants; so, they may be used as feed additives and chemotherapeutics (Citarasu, 2010; Dügenci et al., 2003; Xiang and Zhou, 2000). The use of medicinal plants as natural feed additive in fish diets is useful as a substitute for classical chemotherapeutics, which may have a cumulative effect on fish health. These plants also have growth and immuno-stimulating activities for fish (see Reverter et al., 2014).

Many studies have been conducted to determine the effect of widely consumed coffee bean, *Coffea arabica* on human health. However, it contains many substances such as caffeine, cafestol, kahweol, and chlorogenic acids that show great antioxidant activities (Pellegrini et al., 2003; Vinson et al., 2005). Moreover, coffee and its constituents may improve the defense system against different stressors including heavy metals pollution. In this regard, Lacorte et al. (2013) investigated effects of caffeine (20 mg/L) intake on cadmium (15 mg/L) accumulation in the Wistar rat's blood, testes, epididymis and prostate as well as cadmium-induced changes to the antioxidant defense system of the epididymis. They found that caffeine increased the defense system and reduced the cadmium bioaccumulation in all tissues analyzed.

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Zinc (Zn) has been recognized to play a vital role in almost all aspects of living systems either directly or indirectly (Shukla et al., 2007; Srivastava, 2007). Fish generally require Zn in a certain concentration for desirable fish growth (Watanabe et al., 1997) but its overaccumulation is hazardous to fish (Gupta and Srivastava, 2006; Senthil Murugan et al., 2008). On the other hand, pollution of the aquatic environment by Zn has become a serious health concern in recent years. This metal is introduced into the environment through various routes such as industrials effluents, agriculture pesticide runoff, domestic garbage dumps, and mining activities (Merian, 1991). Among aquatic organisms, fish are generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems (van der Oost et al., 2003). Zinc concentrations in some Egyptian lakes ranged from 0.004 to 0.46 mg/L (Saeed and Shaker, 2008) and it reached 7.94 mg/L in some heavy-polluted lakes (Abdel-Baky et al., 1998).

There are many attempts to use feed supplementation to improve fish growth and immunity but the potential use of medicinal plants to enhance fish resistance to environmental stress is limited. The use of roasted coffee powder (RCP) as a natural feed additive in Nile tilapia diets was established (Abdel-Tawwab, 2015a), but its use to protect fish from heavy metals toxicity has not been evaluated. Common carp, *Cyprinus carpio* L. is one of the widely cultured carp species, which may be commonly found in a wide range of Zn-polluted habitats. Therefore, this study was conducted to evaluate the use of RCP as a natural feed additive to enhance growth, biochemical status, and resistance of common carp to waterborne Zn toxicity.

2. Materials and methods

2.1. Diet preparation, fish culture, and feeding regime

Roasted coffee powder (RCP; *C. arabica*) was obtained from a local market and five different diets containing 0.0, 0.5, 1.0, 2.0, and 5.0 g RCP/kg diet were formulated to contain 30% crude protein (Table 1). However, RCP of each diet was suspended in 100 mL per 1 kg and blended with the other ingredients for 40 min to make a paste of each diet. The pastes were separately passed through a grinder and pelleted through 1-mm diameter paste extruder. The diets were oven-dried at 55 °C for 24 h and stored in plastic bags at −2 °C for further use.

Common carp, *C. carpio* L., fingerlings were obtained from nursery ponds, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were kept in an indoor fiberglass tank for 2 weeks for acclimation to the laboratory conditions. Twenty fish were frozen at −20 °C for chemical analysis at an initial time point. Fish (10.2 ± 0.42 g) were randomly distributed at a rate of 20 fish per aquarium in triplicates, and each aquarium was supplied with compressed air via air-stones using aquarium's air pump. Fish were fed diets up to satiation twice daily at 9:00 and 14:00 h for 10 weeks. Diets were not offered on sampling days. Fish in each aquarium were collected, counted and group-weighed at 2-week intervals. Settled fish waste along with three-quarters of an aquarium's water was siphoned daily, which was replaced by clean and well-aerated water from a storage tank. Fish mortality was recorded daily and dead fish were removed.

After the feeding trial, fish from each treatment were collected and randomly distributed into duplicate 100-L aquaria at a rate of 20 fish per aquarium, and fish were exposed to 5.0 mg Zn/L; the sublethal concentration of Zn was 64.0 mg Zn/L according to Abdel-Tawwab et al. (2013), for 7 days. During the Zn exposure trial, diets were offered to fish up to satiation twice daily at 9:00 and 14:00 h. One half of the aquarium's water along with fish feces and feed remains was siphoned and replaced daily with well-aerated water containing the same Zn concentration. Five fish from each aquarium treatment were collected for determination of Zn residues. The rest of fish were collected and used for biochemical assays.

2.2. Water quality parameters

Water samples were collected biweekly at 15 cm depth from each aquarium to monitor water quality parameters. Dissolved oxygen and water temperature were measured on site using an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, OH, USA). Unionized ammonia was measured using HANNA kits (HANNA Instruments, Rhode Island, USA). The pH was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). Total alkalinity and total hardness were determined by titration according to Boyd (1984). In all treatments, water temperature was 26.3 ± 1.11 °C, dissolved oxygen concentration was 5.8 ± 0.41 mg/L, pH was 7.9 ± 0.09, and unionized ammonia concentration was 0.43 ± 0.016 mg/L. Total alkalinity and total hardness were 141.7 ± 3.1 and 145.6 ± 10.2 mg/L as $CaCO_3$, respectively. All the previous water quality parameters are within the acceptable range for fish growth (Boyd, 1984).

2.3. Growth and feed utilization parameters

Growth performance was determined and feed utilization was calculated as following:

Weight gain $W_2 - W_1$;

Specific growth rate (SGR) $100\,[\ln W_2\,(g) - \ln W_1\,(g)]\,/\,T$; where W_2 is final weight, W_1 is initial weight, and T is the experimental period (day);

Feed conversion ratio (FCR) feed intake / weight gain;

Energy utilization (EU; %) 100 [energy gain in fish / energy intake in feed].

Table 1
Composition and proximate chemical analysis (%; on DM bases) of diets containing different levels of roasted coffee bean.

Ingredients (%)	Roasted coffee bean powder (g/kg diet)				
	0.0 (Control)	0.5	1.0	2.0	5.0
Fish meal	8.5	8.5	8.5	8.5	8.5
Soybean meal	46.5	46.5	46.5	46.5	46.5
Wheat bran	18.3	18.3	18.3	18.3	18.3
Ground corn	10.0	10.0	10.0	10.0	10.0
Corn oil	3.0	3.0	3.0	3.0	3.0
Cod liver oil	3.0	3.0	3.0	3.0	3.0
Mineral mixture[a]	2.0	2.0	2.0	2.0	2.0
Vitamin mixture[b]	2.0	2.0	2.0	2.0	2.0
Starch	6.7	6.2	5.7	4.7	1.7
Coffee grains powder	0.0	0.5	1.0	2.0	5.0
Total	100.0	100.0	100.0	100.0	100.0
Chemical composition (%)					
Dry matter	91.2	91.2	91.3	91.2	91.0
Crude protein	30.2	30.2	30.0	30.1	30.5
Ether extract	8.8	8.6	8.7	8.9	9.1
Crude fiber	4.8	4.8	4.5	4.9	5.0
Ash	6.0	6.0	5.9	6.2	6.1
Zinc concentration (mg/g dry weight)	8.4	8.3	8.1	7.9	8.1
NFE[c]	56.4	56.4	53.9	50.2	46.7
GE (kcal/100 g feed)[d]	479.5	478.4	479	479	481.1

[a] Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[b] Mineral premix (g/kg of premix): $CaHPO_4 \cdot 2H_2O$, 727.2; $MgCO_4 \cdot 7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7 \cdot 3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2 \cdot 4H_2O$, 2.5; $Cu(OAc)_2 \cdot H_2O$, 0.785; $CoCl_3 \cdot 6H_2O$, 0.477; $CaIO_3 \cdot 6H_2O$, 0.295; $CrCl_3 \cdot 6H_2O$, 0.128; $AlCl_3 \cdot 6H_2O$, 0.54; Na_2SeO_3, 0.03.

[c] NFE (nitrogen free extract) = 100 − (protein + lipid + ash + crude fiber).

[d] GE (gross energy): calculated after NRC (1993) as 5.64, 9.44 and 4.11 kcal/g for protein, lipid and carbohydrates, respectively.

2.4. Biochemical measurements

At the end of the experiment (week 10) and after Zn exposure, fish were not fed during the 24 h immediately prior to blood sampling and blood was collected from the caudal vein via heparinized syringe. The collected blood was centrifuged at 5000 ×*g* for 15 min at room temperature. The collected plasma were stored at −20 °C for further assays. Glucose, total protein, total lipids, creatinine, and uric acid in fish plasma were determined colorimetrically according to Trinder (1969), Henry (1964), Joseph et al. (1972), Henry (1974), and Barham and Trinder (1972), respectively. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957). The production of oxygen radicals by leukocytes was assayed by the reduction of Nitro Blue Tetrazolium (NBT, Sigma-Aldrich Chemical, St. Louis, MO, USA) according to Rook et al. (1985). Absorbance was converted to NBT units based on a standard curve of NBT diformazan per milliliter of blood.

2.5. Proximate chemical analyses

The proximate chemical analyses of diets and whole-fish bodies were carried out according to the standard methods of AOAC (1990) for moisture, crude protein, total lipids, and total ash. Moisture content was estimated by drying the samples at 85 °C in a heat-oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) for 48 h. Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco Corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 h. Total ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h.

2.6. Zinc residue

For measuring Zn residues in diets and fish bodies, samples were oven-dried at 85 °C until constant weight and 1.0 g dry weight was ashed in a muffle furnace for 6 h. Ash was digested with 5 ml conc. H_2SO_4 and gradually kept at 130 °C on a hot plate until complete dryness. Then, the digests were diluted with 2 N HCl to a constant volume. Zinc concentration was determined with an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK), which was calibrated using Zn standard solutions.

2.7. Statistical analysis

The results were presented as mean ± SD of three replicates. Prior to statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. The homogeneity of variances among different treatments was tested using Bartlett's test. Then, they were subjected to two-way ANOVA to evaluate effect of RCP supplementation and Zn toxicity. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were done using SPSS program version 15 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

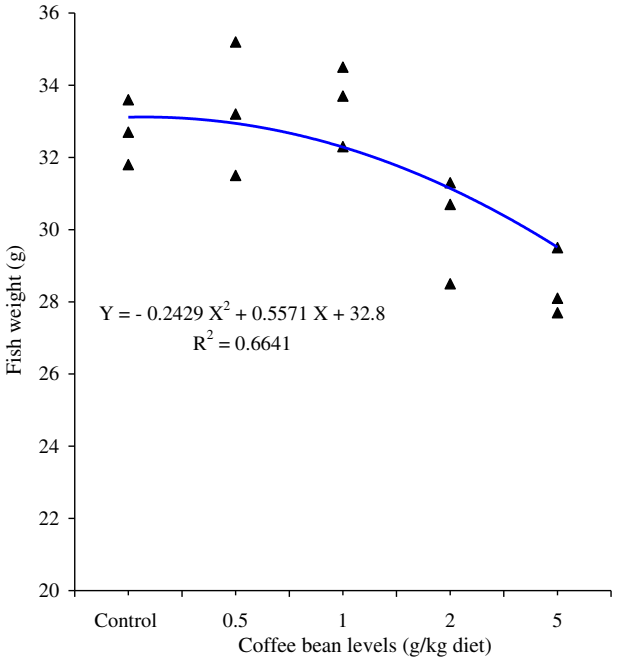

Fig. 1. The relationships between the weight of common carp (g) and different levels of roasted coffee powder in diets. N = 3.

3. Results and discussion

Final fish weight, weight gain, and specific growth rate were not significantly ($P < 0.05$) affected by RCP inclusion in diets up to 1.0 g/kg diet, after which fish growth declined. The lowest fish growth was obtained with 2.0–5.0 g RCB/kg diets. Fish survival range was 93.3–96.7% with no significant difference ($P > 0.05$) among the different treatments (Table 2). The relationship between final weight and RCP levels (Fig. 1) was best expressed by the second-order polynomial regression equations as follows: $Y = -0.2429\ X^2 + 0.5571\ X + 32.8$.

Fish fed diets containing 2.0–5.0 g RCP/kg diet consumed less feed than fish in the other treatments, resulting in highest FCRs (1.46 and 1.53, respectively). In contrast, fish fed 0.0–1.0 RCP/kg diet consumed approximately the same amount of feed (29.7–30.3 g feed/fish), resulting in FCR of 1.30–1.33 (Table 2). Additionally, energy utilization decreased significantly with increasing RCP levels and lowest value was obtained at 5.0 g RCP/kg diet (30.8%; Table 2). Throughout the feeding period fish in all experimental groups were in good health and dose-related mortalities were not observed. This indicates that common carp can tolerate RCP levels up to 5.0 g/kg diet, albeit with reduced growth rate and increased FCR. The adverse effect of coffee-containing diets on fish growth was reported by Fagbenro and Arowosoge (1991), Moreau et al. (2003), and Ulloa and Verreth (2003).

Table 2
Growth performance of common carp fed diets containing different levels of roasted coffee powder for 10 weeks.

Coffee bean (g/kg diet)	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (% g/day)	Feed intake (g feed/fish)	FCR	Energy utilization (%)	Fish survival (%)
0.0	10.3 ± 0.29	32.7 ± 0.52 a	22.4 ± 0.23 a	1.656 ± 0.018 a	30.2 ± 0.36 a	1.33 ± 0.026 b	39.8 ± 0.96 a	96.7 ± 3.33
0.5	10.1 ± 0.17	33.3 ± 1.07 a	23.2 ± 1.01 a	1.703 ± 0.041 a	30.3 ± 0.47 a	1.31 ± 0.061 b	40.5 ± 0.90 a	96.7 ± 3.33
1.0	10.2 ± 0.42	33.5 ± 0.64 a	23.3 ± 0.72 a	1.701 ± 0.063 a	29.7 ± 0.93 a	1.30 ± 0.036 b	38.6 ± 0.90 a	96.7 ± 3.33
2.0	10.2 ± 0.23	30.2 ± 0.85 b	19.9 ± 0.87 b	1.544 ± 0.051 ab	28.9 ± 0.44 ab	1.46 ± 0.053 ab	32.4 ± 0.81 b	96.7 ± 3.33
5.0	10.2 ± 0.25	28.4 ± 0.55 b	18.2 ± 0.79 b	1.465 ± 0.062 b	27.8 ± 0.35 b	1.53 ± 0.068 a	30.8 ± 0.73 b	93.3 ± 3.33

Means followed by the same letter in the same column are not significantly different at $P < 0.05$.

Table 3
Proximate analysis of common carp (%; on fresh weight basis) of common carp fed diets containing different levels of roasted coffee powder for 10 weeks.

Coffee bean (g/kg diet)	Moisture	Crude protein	Total lipids	Total ash
0.0	66.6 ± 1.44	20.8 ± 0.22 a	7.5 ± 0.38 a	5.0 ± 0.20 b
0.5	66.7 ± 3.19	20.6 ± 0.20 a	7.5 ± 0.16 a	5.0 ± 0.05 b
1.0	67.9 ± 2.28	20.0 ± 0.54 ab	6.7 ± 0.52 ab	5.3 ± 0.22 b
2.0	67.2 ± 4.54	19.5 ± 0.10 b	5.8 ± 0.31 bc	5.4 ± 0.23 b
5.0	67.2 ± 2.27	19.4 ± 0.33 b	5.7 ± 0.23 c	6.5 ± 0.19 a

Means followed by the same letter in the same column are not significantly different at $P < 0.05$.

The obtained results suggest that growth retardation at 2.0–5.0 g RCP/kg diet may be due to low feed intake and low energy utilization, which may be possibly because of its bitter taste (Frank et al., 2004; Mazzafera, 2002). In this concern, Ulloa and Verreth (2003) reported that caffeine in coffee, together with polyphenols and tannins could deter feed consumption by fish. Kasumyan and Døving (2003) reported that caffeine inhibited the feeding behavior of turbot, *Psetta maxima*. Lamb and Finger (1995) found that goldfish (*Carassius auratus*) disliked caffeine supplementation at concentrations >2.5 mg. Chatzifotis et al. (2008) reported that sea bream, *Sparus aurata* did not accept a caffeine-containing diet at 10 g/kg, but at doses ≤5 g/kg caffeine appeared to have no detrimental effect. Abdel-Tawwab (2015a) found that incorporating RCP in Nile tilapia diets reduced their feed intake at levels >1.0 g/kg diet.

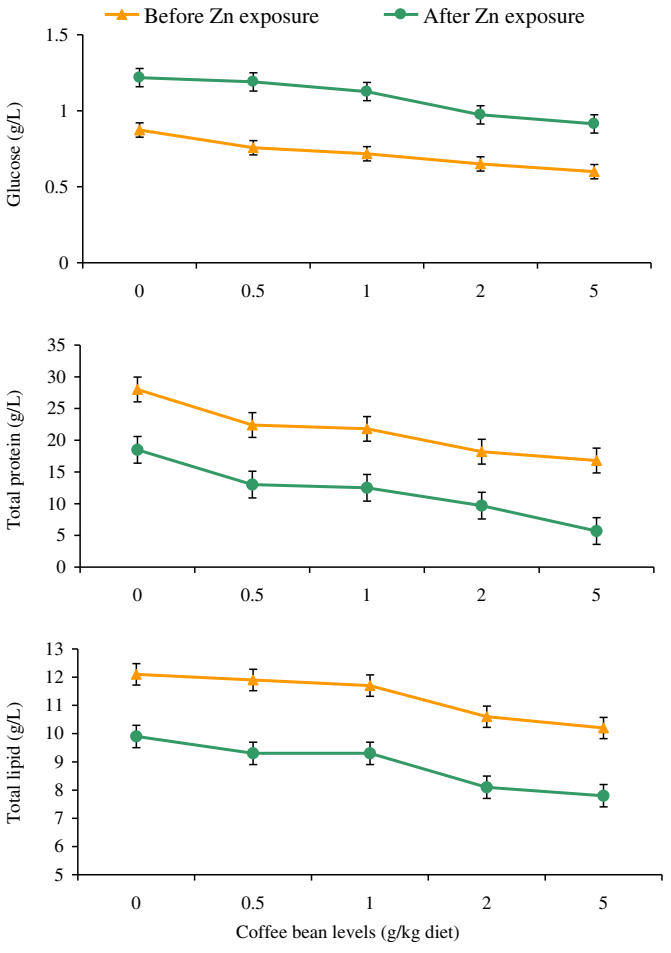

Fig. 2. Changes in plasmatic glucose (mg/L), total protein (g/L), and total lipids (g/L) of common carp fed different levels of roasted coffee powder for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days.

The RCP supplementation affected significantly the whole-fish body composition only at highest inclusion levels resulting in lowest contents of protein and total lipids (19.4 and 5.7%, respectively) and highest ash content (6.5%) at 5.0 g RCP/kg diet (Table 3). These results may be because of low energy utilization at 2.0–5.0 g RCP/kg diets. And fish used body protein and lipids as energy sources for different biochemical functions. Similar results were found by Abdel-Tawwab (2015a) who reported that RCP supplementation increased lipid content and decreased protein content in Nile tilapia body. Kobayashi-Hattori et al. (2005) reported that caffeine induced lipolysis reducing body fat in rats fed a high-fat diet. Contrarily, Chatzifotis et al. (2008) found that caffeine cannot reduce the lipid content of white muscle and liver in sea bream. Moreover, the changes in protein and lipid contents in fish body could be linked with changes in their synthesis and/or deposition rate in fish body (Abdel-Tawwab et al., 2006; Fauconneau, 1984; Smith, 1981).

Prior to Zn exposure, plasmatic glucose, total protein, and total lipids levels decreased significantly ($P > 0.05$; Fig. 2), meanwhile AST, ALT, creatinine, and uric acid levels increased significantly with increasing RCP levels ($P > 0.05$; Figs. 3–4). These results suggest that high RCP levels stressed the overall fish health. In contrast to the present study, Dügenci et al. (2003) reported that serum total protein level in rainbow trout increased significantly after feeding fish with various herbal extracts. Moreover, Abdel-Tawwab (2015b), Ahmad et al. (2011), and Abdel-Tawwab et al. (2010a) found improvements in health and immunity of Nile tilapia fed diets containing American ginseng, *Panax quinquefolium*, green tea, cinnamon, *Cinnamomum zeylanicum*, respectively. Moreover, the decrease in blood protein and lipids would result when catabolic processes exceeded anabolic ones to meet increased metabolic requirements of fish.

It is also noticed that NBT increased significantly with increasing RCP levels at 1.0–5.0 g/kg diet ($P < 0.05$; Fig. 5). This result suggests that RCP has an immunostimulant effect. The mechanism of immunostimulation of dietary RCP may be attributed to one or more of its constitutes especially caffeine, cafestol, kahweol, and chlorogenic acid that show antioxidant activities (Pellegrini et al., 2003; Vinson et al., 2005). These substances have powerful natural antioxidants (Farhoosh et al.,

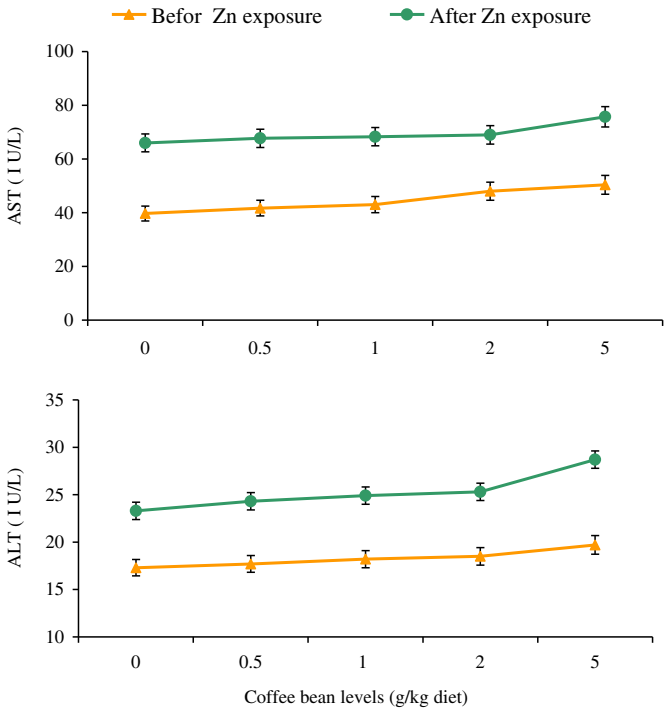

Fig. 3. Changes in plasmatic AST (IU/L) and ALT (IU/L) of common carp fed different levels of roasted coffee powder for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days.

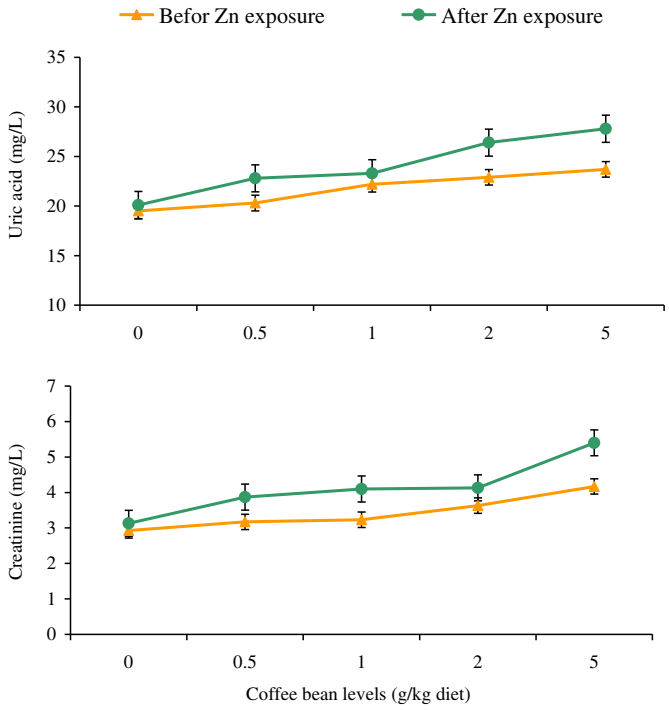

Fig. 4. Changes in plasmatic uric acid (mg/L) and creatinine (mg/L) of common carp fed different levels of roasted coffee powder for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days.

2007; Rusak et al., 2008; Wu et al., 2007). The usefulness of antioxidants in protecting cellular components against oxidative stress is well established (Mohan et al., 2006).

Post-Zn exposure, biochemical variables and Zn residues in fish were significantly affected by RCP supplementation, Zn exposure, and their interactions ($P < 0.05$; Figs. 2–5). However, Zn effect was more severe in fish fed a RCP-free diet than those fed RCP-enriched diets. The highest values of glucose (1.218 g/L), total protein (18.5 g/L), and total lipids (9.9 g/L), meanwhile the lowest values of AST (66.0 IU/L), ALT (23.3 IU/L), creatinine (3.13 mg/L), and uric acid (20.1 mg/L) were detected in Zn-toxicated control fish as compared with Zn toxicated and RCP-fed fish. Moreover, NBT decreased significantly due to Zn toxicity in RCP-fed fish (Fig. 5).

The high glucose value in Zn-toxicated control fish suggests a stress susceptibility of fish against Zn toxicity. This hyperglycemia may be attributed to cortisol-mediated glycogenolysis or gluconeogenesis (Mommsen et al., 1999). The primary response against stress involves the increases in plasma cortisol (Barton, 2002; Barton and Iwama,

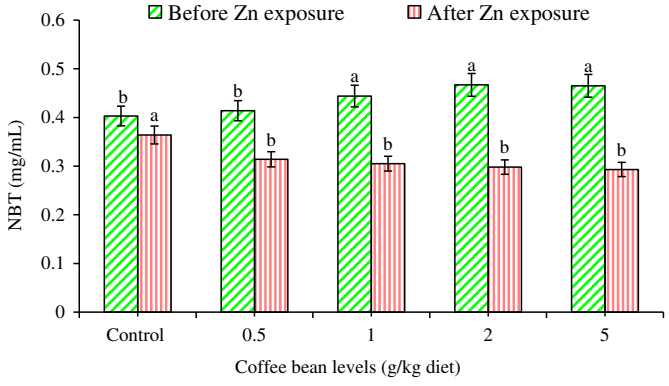

Fig. 5. Plasmatic nitroblue tetrazolium (NBT; mg/mL) of common carp fed different levels of roasted coffee powder for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days. The same bars assigned with the same letter are not significantly differed at $P < 0.05$.

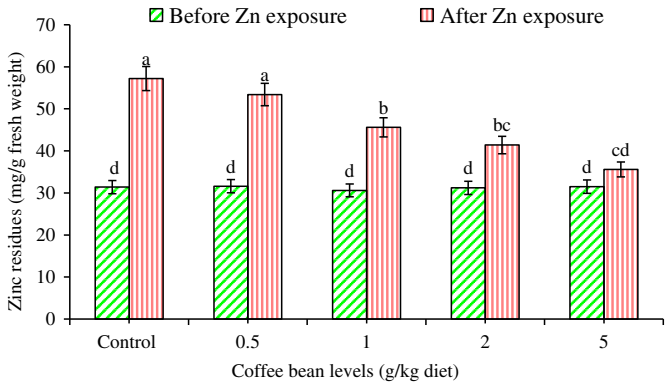

Fig. 6. Zinc residues (mg/g fresh weight) in whole body of common carp fed different levels of roasted coffee powder for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days. The same bars assigned with the same letter are not significantly differed at $P < 0.05$.

1991). This hormone induces secondary stress responses, characterized by increased glucose levels, mobilizing glucose to tissues for homeostasis to cope with energy-demanding processes of restoration (Barton et al., 2002; Wendelaar Bonga, 1997). These results agree with Firat and Kargin (2010) who found an increase in glucose due to Zn, Cd, and Zn + Cd exposure in Nile tilapia. Abdel-Tawwab et al. (2012, 2013) found increases in glucose levels in Nile tilapia and common carp, respectively due to Zn toxicity.

In addition, plasmatic total protein and total lipids values in Zn-toxicated control fish were significantly lower than non-toxicated fish. In Zn-exposed fish fed RCP-enriched diets, values of glucose, protein, and lipids decreased significantly, meanwhile AST, ALT, creatinine, and uric acid increased significantly with increasing RCP levels in fish diets ($P > 0.05$). Moreover, after Zn exposure, Zn residues in whole-fish body decreased significantly with increasing RCP levels in fish diets and lowest Zn content was detected in fish fed 5.0 g RCP/kg diet (47.2 mg Zn/g dry weight; Fig. 6). The relationship between dietary RCP levels and daily Zn accumulation in whole-fish body (Fig. 7) was

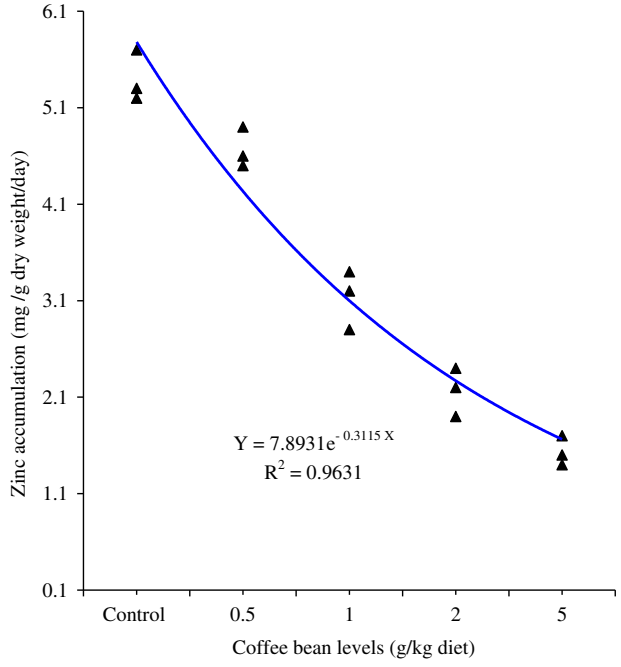

Fig. 7. The relationships between daily Zn accumulation (mg/g dry weight/day) in whole body of common carp and different levels of roasted coffee powder in diets. N = 3.

best expressed by the second-order polynomial regression equations as follows: $Y = 7.8931\ e^{-0.3115\ X}$.

These results suggested that RCP supplementation may have played a role in reducing Zn toxicity. However, coffee has been reported to have strong antioxidant activity with a high capacity for scavenging superoxide radicals (Pellegrini et al., 2003; Vinson et al., 2005). Therefore, RCP may likely protect cultured fish from the adverse effects of Zn and reduced the Zn level via metal–ion chelation, increasing metal excretion, and/or decreasing metal absorption. In similar study, Abdel-Tawwab (2015b) found that American ginseng supplementation reduced copper (Cu) toxicity for Nile tilapia. Abdel-Tawwab et al. (2007) used organic selenium (OS) supplementation to resist Cu toxicity by African catfish. They found that the supplementation of 0.3 g OS/kg diet could reduce significantly Cu residue in fish body. Abdel-Tawwab and Wafeek (2010) concluded that the supplementation of 0.5 g OS/kg diet may reduce the harmful effect of waterborne Cd on Nile tilapia where OS reduced significantly Cd residues in fish body. Abdel-Tawwab et al. (2010b) evaluated the resistance of Galilee tilapia to waterborne Cu toxicity when fed live baker yeast. They found that the inclusion of 10 g baker yeast/kg diet reduced the Cu absorption and accumulation in whole-fish body.

It could be concluded from the present study that the inclusion of RCP in common carp diets could not improve fish growth and feed utilization but it could reduce Zn toxicity.

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