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Interactive effects of coffee bean supplementation and waterborne zinc toxicity on growth performance, biochemical variables, antioxidant activity and zinc bioaccumulation in whole body of common carp, *Cyprinus carpio* L.

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Abstract

Roasted coffee powder (RCP; *Coffea arabica*) is usually used as a beverage for human but there are few attempts to use it as a natural feed supplement in fish diets. In this study, common carp, *Cyprinus carpio* L., (11.8 ± 0.09 g) were reared in zinc (Zn)-containing water at concentrations of 0.0 or 5.0 mg/L and cosupplemented with 0.0 or 1.0 g RCP/kg diet for 6 weeks to investigate effects of RCP supplementation, Zn exposure and their interaction on fish performance, biochemical variables, antioxidant activity and Zn bioaccumulation in whole fish body. Fish growth and feed intake were significantly affected by RCP supplementation, Zn toxicity and their interaction. However, fish fed a RCP-supplemented diet did not exhibit better performance than those fed the RCP-free diet and both diets produced higher fish performance than the Zn-toxicated fish. It is noticed that RCP supplementation to Zn-toxicated fish enhanced their growth, and feed utilization as compared to Zn-toxicated fish alone. Fish fed control and RCP-enriched diets showed no significant differences in biochemical variables, which were significantly altered due to waterborne Zn toxicity. Moreover, Zn reduced significantly; meanwhile, RCP supplementation increased significantly superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities. Notably, Zn exposure could reduce fish growth and antioxidant activity and increase Zn deposition in whole fish body. And RCP intake could enhance the antioxidant activity exerting a protective effect against Zn toxicity, thereby reducing Zn bioaccumulation in whole fish body.

KEYWORDS

antioxidant activity, biochemical variables, coffee bean, common carp, fish performance, Zn bioaccumulation, Zn toxicity

1 | INTRODUCTION

Zinc (Zn) is an essential element which plays a vital role in almost all aspects of living systems; it serves as an intracellular signalling agent, as an antioxidant, and a vital constituent of many enzymes (Hogstrand, 2012; Srivastava, 2007). Fish generally require Zn in a certain concentration for desirable growth (Watanabe, Kiron, & Satoh, 1997), but its over-accumulation is hazardous to fish (Gupta & Srivastava, 2006; Senthil Murugan, Karuppasamy, Poongodi, & Puvaneswari, 2008). Among aquatic organisms, fish are generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems (Fazio et al., 2014; van der Oost, Beyer, & Vermeulen, 2003).

However, at higher Zn concentrations, physiological and biochemical mechanisms may be disrupted (Gioda et al., 2007; Hogstrand, 2012; Loro, Jorge, da Silva, & Wood, 2012; Loro, Nadella, & Wood, 2014).

Common carp, *Cyprinus carpio* L., is one of the widely cultured carp species all over the world and it may be commonly found in a wide range of Zn-polluted aquatic ecosystems. Generally, Zn is introduced into aquatic systems through industrial process and agriculture fertilizers (see Eisler, 1993; Merian, 1991). It is known that Zn exposure could cause significant alterations in fish physiology resulting in retardation in their growth and feed utilization (Abdel-Tawwab, 2016; Abdel-Tawwab, El-Sayed, & Shady, 2012, 2016; Carbone & Faggio 2016; Srivastava, 2007). There are many attempts to use feed supplements to improve fish growth and immunity (Abdel-Tawwab & Abbass, 2017; Guardiola et al., 2016) but their potential use to enhance fish resistance against environmental stress is limited (Abdel-Tawwab, 2015b; Abdel-Tawwab, Sharafeldin, Mousaad, & Ismaiel, 2015; Faggio, Fazio, Marafioti, Arfuso, & Piccione, 2015).

Arabic coffee bean, *Coffea arabica*, contains many bioactive compounds such as caffeine, cafestol, kahweol and chlorogenic acids those show great antioxidant activities (Cho et al., 2009; Kitzberger, Scholz, & Benassi, 2014; Noschang et al., 2009; Sánchez-González, Jimenez-Escrig, & Saura-Calixto, 2005). Roasted coffee powder (RCP) is generally used as a beverage for human but its potential use as a feed additive in fish diets is still limited (Abdel-Tawwab, 2015a; Abdel-Tawwab et al., 2015). In our previous study, Abdel-Tawwab et al. (2015) fed common carp with diets containing RCP levels prior to Zn exposure to assess the capability of RCP feeding history to overcome the sudden waterborne Zn exposure but the combination of RCP supplementation with water Zn exposure needs to be explored. Therefore, this study was conducted to evaluate the combined effects of RCP supplementation with waterborne Zn toxicity on the biochemical status, the antioxidant activity and the performance of common carp. Zinc bioaccumulation in whole fish body was also evaluated.

2 | MATERIALS AND METHODS

2.1 | Diet preparation, fish culture and feeding regime

The experiment was based on a 2 × 2 factorial design with two levels of supplemented coffee powder (0.0 or 1.0 g/kg diet) and two waterborne Zn concentrations (0.0 or 5.0 mg/L) in triplicates. Roasted coffee bean (RCP; *C. arabica*) was obtained from a local market; it contains 59 g moisture, 86 g crude protein, 112 g total lipids and 72 g total ash per kilo beans. Two different diets containing 0.0 (control) or 1.0 g RCP/kg diet were formulated to contain 300 g crude protein per kilo feed. (Table 1). Roasted coffee powder was suspended in 100 ml per one kg and blended with the other diet ingredients for 40 min to make a paste. 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 hr 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 fibreglass tank for 2 weeks for acclimation to the laboratory conditions during which fish fed on the control diet. Twenty fish were frozen at −20°C for chemical analysis at an initial time point. Fish (11.8 ± 0.09 g) were randomly distributed at a rate of 20 fish per 100-L aquarium in triplicates. Each aquarium was supplied with compressed air via air stones using aquarium's air pumps.

Zinc sulphate ($ZnSO_4.7H_2O$, Merck & Co Inc., Kenilworth, NJ, USA) was dissolved in distilled water at a rate of 10 g Zn/L as a stock solution and an appropriate volume was added to Zn-exposed aquaria to obtain the nominal concentration of 5.0 mg Zn/L. Other aquaria were Zn-free and were used as controls. Under each Zn concentration, that is 0.0 or 5.0 mg Zn/L, fish fed 0.0 or 1.0 g RCP/kg diet up to satiation twice daily at 9:00 and 14:00 hr for 6 weeks. Fish in each aquarium

TABLE 1 Composition and proximate chemical analysis (%; on dry matter basis) of diets containing different levels of roasted coffee powder

	Roasted coffee powder (g/kg diet)	
Ingredients	**0.0 (Control)**	**1.0**
Fish meal	85	85
Soybean meal	465	465
Wheat bran	183	183
Ground corn	100	100
Corn oil	20	20
Cod liver oil	20	20
Mineral mixture[a]	20	20
Vitamin mixture[b]	20	20
Starch	87	86
Coffee bean powder	0.0	1.0
Total	1,000	1,000
Chemical composition (g/kg)		
Dry matter	912	913
Crude protein	302	300
Total lipids	88	87
Crude fibre	48	45
Total ash	60	59
Zinc concentration	8.4	8.1
Nitrogen free extract (NFE)[c]	564	539
Gross energy (kcal/kg diet)[d]	4,852	4,729

[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): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$, 127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; $Cu(OAc)_2.H_2O$, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03.
[c]Nitrogen free extract = 1000 − (g/kg of protein + lipid + ash + crude fibre).
[d]Gross energy: Calculated after NRC (National Research Council) (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and NFE, respectively.

were collected, counted and group-weighed at 2-week intervals. Diets were not offered on sampling days. Settled fish wastes were siphoned daily together with a half of the water in each aquarium and replaced daily with well-aerated water containing the same Zn concentration. Dead fish were removed, and fish survival was recorded. The water temperature during the running of this experiment was 27.5 ± 0.3°C with 12-hr: 12-hr light–dark photoperiod cycle using fluorescent tubes as a light source. At the end of the experiment, five fish from each aquarium were collected for determination of Zn residues and the rest of fish were collected and used for biochemical assays.

2.2 | Water quality parameters

Water samples were collected weekly at 15 cm depth from each aquarium, and water quality parameters were monitored. Water temperature and dissolved oxygen were measured in site using a portable oxygen meter (Jenway, London, UK). 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). It was found that dissolved oxygen concentration was 5.5 ± 0.1 mg/L, and pH was 8.1 ± 0.09. Total alkalinity and total hardness were 145.4 ± 7.8 and 147.5 ± 3.6 mg/L as $CaCO_3$, respectively. These parameters ranges are suitable for fish growth (Boyd, 1984).

2.3 | Growth parameters and feed utilization

At the end of feeding trial, fish of each aquarium were collected, counted and group-weighed. Parameters of growth performance and feed utilization were calculated as follows:

$$\text{Weight gain} = W_2 - W_1;$$

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

Feed intake is the summation of feed offered to fish during the whole experimental period;

$$\text{Feed conversion ratio (FCR)} = \text{feed intake/weight gain};$$

$$\text{Energy utilization (EU; \%)} = 100\,[\text{energy gain/energy intake}].$$

2.4 | Biochemical measurements

At the end of the experiment, fish were not fed during the 24 hr prior to blood sampling and blood was collected from the caudal vessel via heparinized syringes. The collected blood was centrifuged at 10,000 × *g* for 15 min at room temperature. The collected plasma was stored at −20°C for further assays. Aliquots were used only once for each assay and were not frozen again for re-analysis to prevent or minimize contamination. Plasmatic glucose, total protein, total lipids, creatinine and uric acid were determined colorimetrically according to Trinder (1969), Henry (1964), Joseph, Knight, Anderson, James, and Rawie (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 antioxidant enzymes activities in fish plasma were measured using the diagnostic reagent kits according to the manufacturer's instructions. Superoxide dismutase (SOD) activity was measured at 440 nm (Marklund & Marklund, 1974). Catalase (CAT) activity was estimated at 240 nm following the method of Aebi (1984). Activity of glutathione peroxidase (GPx) was measured at 340 nm according to Paglia and Valentine (1967).

2.5 | Zinc residue

For measuring Zn residues in diets and fish body, samples were oven-dried at 85°C until constant weight and 1.0 g dry weight was ashed in a muffle furnace for 6 hr. Ash was digested with 5 ml concentrated 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. After that, Zn concentration was determined with an atomic absorption spectrophotometer (Thermo 6600; Thermo Electron Corporation, Cambridge, UK), which was calibrated using Zn standard solution.

2.6 | 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 variance among different treatments was tested using Bartlett's test. Then, the data were subjected to two-way ANOVA to evaluate effects of RCP supplementation and Zn toxicity. Differences between means were tested at the 5% probability level using Duncan test. All the statistical analyses were performed using SPSS program version 15 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

3 | RESULTS

In the present study, fish growth and feed intake were significantly ($p < .05$) affected by RCP supplementation, Zn toxicity and their interaction (Table 2). It is noticed that RCP supplementation did not improve fish performance over those fed the control diet (T1 versus T3); both diets produced higher fish growth than the other treatments. Fish exposed to Zn alone (T2) exhibited lowest growth; meanwhile, RCP supplementation in combination with Zn toxicity reduced significantly the adverse effect of Zn toxicity (T2 versus T4; Table 2). Additionally, fish fed control or RCP-enriched diets (T1 and T3) consumed more feed (32.7 ± 0.19 and 33.2 ± 0.17 g feed/fish, respectively) and energy (156.5 ± 0.97 and 159.0 ± 0.73 Kcal/fish, respectively) than Zn-toxicated fish groups (T2 and T4) resulting in FCRs of 1.42 (Table 2). Lowest feed intake was observed in Zn-toxicated fish (T2; 17.4 ± 0.13 g feed/fish) resulting in highest FCR (1.71 ± 0.042). Additionally, RCP cosupplementation to Zn-toxicated fish (T4) enhanced their feed and energy intakes as compared to Zn-toxicated

TABLE 2 Growth performance of common carp fed diets containing different levels of roasted coffee powder (RCP) and exposed to either 0.0 or 5.0 mg Zn/L for 6 weeks

Treatments		Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (%g/day)	Feed intake (g feed/fish)	FCR	Energy intake (Kcal/fish)	Fish survival (%)
Control	T1	11.8 ± 0.09	34.8 ± 0.75 a	23.0 ± 0.84 a	2.575 ± 0.071 a	32.7 ± 0.19 a	1.42 ± 0.067 b	156.5 ± 0.97 a	100.0 ± 0.00
Control + Zn	T2	11.9 ± 0.09	22.1 ± 0.26 c	10.2 ± 0.24 c	1.474 ± 0.027 c	17.4 ± 0.13 c	1.71 ± 0.042 a	83.0 ± 0.73 c	95.5 ± 2.23
RCP	T3	11.9 ± 0.06	35.3 ± 0.48 a	23.4 ± 0.34 a	2.588 ± 0.008 a	33.2 ± 0.17 a	1.42 ± 0.013 b	159.0 ± 0.73 a	97.8 ± 2.23
RCP + Zn	T4	11.9 ± 0.15	28.1 ± 0.10 b	16.2 ± 0.57 b	2.046 ± 0.044 b	21.4 ± 0.16 b	1.32 ± 0.064 c	102.5 ± 0.83 b	95.5 ± 2.23
Two-way ANOVA		*p* Value							
RCP		.875	.001	.001	.001	.001	.073	.0001	.580
Zn exposure		.640	.001	.001	.001	.001	.007	.0001	.122
RCP x Zn exposure		.640	.001	.010	.001	.001	.053	.0001	.580

Means followed by different letters in the same column are significantly different at $p < .05$.

fish alone (T2 versus T4). Fish survival range was 95.5%–100% with no significant difference ($p > .05$) among the different treatments (Table 2).

Biochemical variables in fish plasma were significantly affected by RCP supplementation, waterborne Zn exposure and their interaction ($p < .05$; Table 3). There were no significant differences in biochemical variables in fish fed control and RCP-enriched diets (T1 versus T3; $p > .05$). Meanwhile, Zn-toxicated fish (T2) produced highest values of plasmatic glucose, AST, ALT, creatinine and uric acid (1.143 ± 0.078 g/L, 45.6 ± 1.85 IU/L, 30.5 ± 1.97 IU/L, 4.7 ± 0.26 mg/L and 28.7 ± 0.72 mg/L, respectively) and lowest values of plasmatic total protein and total lipids (15.2 ± 1.39 and 8.4 ± 0.49 g/L, respectively). It is also noticed that RCP supplementation reduced the impact of Zn toxicity on biochemical variables as compared to the Zn-toxicated fish alone (T2 versus T4; Table 3).

The antioxidant activity indicated by plasmatic SOD, CAT and GPx activities were significantly affected by RCP supplementation, waterborne Zn toxicity and their interaction ($p < .05$; Table 4). Zinc toxicity reduced significantly ($p < .05$); meanwhile, RCP supplementation induced significantly ($p < .05$) SOD, CAT and GPx activities. The RCP cosupplementation with Zn toxicity (T4) showed better antioxidant enzymes activities than those of Zn-toxicated alone (T2).

In Zn-toxicated fish group (T2), Zn residue in whole fish body (27.7 ± 1.74 mg Zn/g fresh weight) was significantly higher than those of the other treatments ($p < .05$; Table 4). Lowest Zn residues (8.1 ± 0.18 and 7.9 ± 0.70 mg Zn/g fresh weight) in the whole fish body were obtained at control and RCP-fed fish (T1 and T3, respectively). It is also noticed fish fed RCP-supplemented diet with Zn toxicity (T4) showed lower Zn residue than Zn-toxicated fish alone (T2).

4 | DISCUSSION

In the present study, RCP supplementation did not improve fish performance and feed utilization over the control diet (T1 versus T3). This may be possibly because of its bitter taste (Frank, Bouverat, MacKinnon, & Hettinger, 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 behaviour of turbot, *Psetta maxima*. These results are in agreement with Abdel-Tawwab (2015a) and Abdel-Tawwab et al. (2015) who found that incorporating RCP in practical diets did not improve growth performance of Nile tilapia and common carp, respectively. Furthermore, Chatzifotis et al. (2008) evaluated effects of dietary caffeine (the main component of coffee) on growth performance of gilthead sea bream (*Sparus aurata*). They found that caffeine adversely affected fish growth at a concentration higher than 1.0 g/kg diet. On the other hand, Zn toxicity reduced fish growth and feed utilization.

The obtained results also indicated that Zn exposure retarded fish performance and feed utilization. These results may be because Zn toxicity impaired normal physiological functions resulting in reduced

TABLE 3 Changes in some plasmatic biochemical parameters of common carp fed diets containing different levels of roasted coffee powder (RCP) and exposed to either 0.0 or 5.0 mg Zn/L for 6 weeks

Treatments		Glucose (g/L)	Total protein (g/L)	Total Lipids (g/L)	AST (IU/L)	ALT (IU/L)	Creatinine (mg/L)	Uric acid (mg/L)
Control	T1	0.800 ± 0.251 c	22.7 ± 0.23 a	14.9 ± 0.42 a	27.8 ± 1.51 c	15.4 ± 0.58 c	3.1 ± 0.12 c	17.1 ± 0.22 c
Control + Zn	T2	1.143 ± 0.078 a	15.2 ± 1.39 c	8.4 ± 0.49 c	45.6 ± 1.85 a	30.5 ± 1.97 a	4.7 ± 0.26 a	28.7 ± 0.72 a
RCP	T3	0.839 ± 0.114 c	21.7 ± 1.10 a	15.6 ± 1.36 a	25.4 ± 1.33 c	15.1 ± 0.22 c	3.0 ± 0.06 c	17.4 ± 1.24 c
RCP + Zn	T4	0.925 ± 0.289 b	18.4 ± 0.43 b	12.3 ± 0.72 b	38.8 ± 1.39 b	23.2 ± 0.92 b	3.9 ± 0.06 b	20.6 ± 0.41 b
Two-way ANOVA		*p* Value						
RCP		0.001	0.006	0.006	0.009	0.001	0.001	0.001
Zn exposure		0.001	0.001	0.001	0.001	0.001	0.001	0.001
RCP x Zn exposure		0.002	0.035	0.016	0.034	0.002	0.001	0.001

Means followed by different letters in the same column are significantly different at $p < .05$.

TABLE 4 Changes in plasmatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities and Zn residuals in whole body of common carp fed diets containing different levels of roasted coffee powder (RCP) and exposed to either 0.0 or 5.0 mg Zn/L for 6 weeks

Treatments		SOD (U/L)	CAT (U/L)	GPx (U/L)	Zn residuals (mg/g fresh weight)
Control	T1	73.8 ± 1.65 b	62.4 ± 1.21 b	46.7 ± 1.65 b	8.1 ± 0.18 c
Control + Zn	T2	63.7 ± 4.21 d	52.1 ± 1.61 d	39.5 ± 1.43 c	27.7 ± 1.74 a
RCP	T3	90.9 ± 4.96 a	65.9 ± 2.04 a	51.9 ± 1.41 a	7.9 ± 0.70 c
RCP + Zn	T4	71.2 ± 1.61 c	58.5 ± 1.64 c	40.8 ± 1.64 c	23.2 ± 1.11 b
Two-way ANOVA		*p* Value			
RCP		0.003	0.012	0.017	0.001
Zn exposure		0.006	0.017	0.015	0.001
RCP x Zn exposure		0.016	0.025	0.018	0.001

Means followed by different letters in the same column are significantly different at $p < .05$.

feed intake and subsequently lowered fish growth (Abdel-Tawwab, 2016; Abdel-Tawwab et al., 2012, 2016). An alternative hypothesis is that due to the reduced feed intake, the required energy to cope Zn toxicity stress was met via the decomposition of the storage-deposited nutrients. Similar results were obtained by Mohanty, Adhikari, Mohanty, and Sarangi (2009) who concluded that the culture of Indian major carp, *Cirrhinus mrigala*, in an environment containing more than 0.06 mg Zn/L could significantly lower their growth and feed intake. Abdel-Tawwab et al. (2012, 2016); Abdel-Tawwab, Mousaad, Sharafeldin, and Ismaiel (2013) also reported that Zn toxicity leads to reduced growth and feed utilization of Nile tilapia and common carp, respectively.

Fish fed control and RCP-enriched diets (T1 and T3) showed no significant differences in biochemical variables, which were significantly affected by waterborne Zn exposure. These results suggest that RCP supplementation did not adversely affect the overall fish health. Meanwhile, Zn toxicity like other aquatic pollution induced several pathological processes in different fish organs (van Dyk, Pieterse, & van Vuren, 2007; Gupta & Srivastava, 2006; Lee et al., 2014) resulting in significant alterations in their physiological and biochemical functions. Furthermore, the obtained results reflect the loss of protein and lipid levels in the Zn-exposed fish, which may be due to increased protein oxidation with Zn exposure (Cakmak, Dogan, & Severcan, 2006; Takahashi, French, & Wong, 1991) and/or the use of those molecules as energetic substrates to cope with stress metabolically (Vijayan, Cristina Pereira, Grau, & Iwama, 1997). Similar results were found with Nile tilapia (Abdel-Tawwab, 2016; Abdel-Tawwab et al., 2012, 2016) and common carp (Abdel-Tawwab et al., 2013) exposed to waterborne Zn toxicity. Also, Gioda et al. (2007) reported that although Zn is required as microelements in the cells, the sublethal Zn concentrations could change biochemical parameters which may alter normal cellular function. Zheng et al. (2011) reported that waterborne Zn toxicity influenced significantly hepatic intermediatory enzymes activities in Javelin Goby, *Synechogobius hasta*.

Fish are able to live in different aquatic ecosystems including metal-contaminated environments and they exert metabolic mechanisms that help them for adaptation (Casella et al., 2012; Faggio, Fedele, Arfuso, Panzera, & Fazio, 2014; Fazio et al., 2014; Stephensen et al., 2000), for detoxification (Pagano et al., 2016; Sheehan, Meade, Foley, & Dowd, 2001) and for the antioxidant protection (Geret & Bebianno, 2004). SOD is an important defence enzyme that catalyses

the dismutation of O_2, which produces H_2O_2 that is converted to H_2O and molecular oxygen by CAT (Qu et al., 2014). Although CAT activity has been inhibited by Zn, this inhibited activity may be correlated with the displacement of endogenous metals (cofactors from the active sites) by Zn. However, the diminished CAT activity suggests that most of the H_2O_2 produced had been neutralized by available CAT and GPx. GPx is a known H_2O_2 scavenger and it plays an important role in the detoxification of reactive oxygen species (ROS; Matés, 2000; Shelly, 2009). Thus, the lower levels of SOD, CAT and GPx activities in Zn-exposed fish are indicative for cell damage due to the accumulation of the high-level free radicals. Similarly, Loro et al. (2012) found that Zn exposure reduced SOD and CAT activities in all tissues of Atlantic killifish, *Fundulus heteroclitus*. They also found that total oxidative scavenging capacity was depleted in Zn-exposed fish. Qu et al. (2014) found that SOD, CAT and GPx activities decreased significantly with short-term Zn exposure but they were gradually recovered to control levels after 30 day suggesting a possible adaptive response to the harmful environment after long-term Zn exposure. On the other hand, Zheng et al. (2011) found increases in SOD and CAT activities in the liver of Javelin Goby, *S. hasta*, due to waterborne Zn exposure. The RCP supplementation herein exhibited protective antioxidant action against cellular damage induced by Zn toxicity. Undoubtedly, these beneficial effects of RCP on antioxidant properties may be due to the presence of active compounds especially caffeine. Caffeine has been reported as a protective substance on cellular damage with beneficial antioxidant effects probably due to its main metabolites, 1-methylxanthine and 1-methyluric acid, that are highly effective antioxidants (Lee, 2000). Additionally, coffee phenolic compounds, such as caffeic acid and chlorogenic acid, showed beneficial effects on the attenuation of oxidative stress (Cho et al., 2009; Huang, Paulis, & May, 2004; Lee & Zhu, 2006). Kahweol and cafestol, two coffee diterpenes, also contain antioxidant properties, especially in the glutathione homeostasis (Huber et al., 2002; Lee & Jeong, 2007).

The high Zn residue in the whole body of Zn-toxicated fish (T2 and T4) is much more expected. Similar results were obtained by Mohanty et al. (2009) who conclude that Zn accumulation in the whole body of Indian major carp increased with increasing Zn concentrations. Abdel-Tawwab et al. (2012, 2016, 2013) also found that Zn accumulation increased significantly in whole bodies of Nile tilapia and common carp, due to Zn toxicity. Zheng et al. (2011) reported that waterborne Zn toxicity increased significantly Zn accumulation in different tissues of Javelin Goby, *S. hasta*. Loro et al. (2014) found reasonable increases in total Zn concentrations in different tissues of killifish, *F. heteroclitus* due to Zn toxicity. Qu et al. (2014) stated that Zn deposition in goldfish, *Carassius auratus*, increased with increasing Zn levels.

It is also noticed that various biochemical parameters in Zn-toxicated fish cosupplemented with RCP-containing diet (T4) were improved towards those of Zn-free groups (T1 and T3). Also, RCP cosupplementation lowered the adverse effect of Zn toxicity and Zn residue in whole fish body (T4 versus T2). These results suggest that RCP supplementation may have played a role in reducing Zn toxicity and improving fish health. These results may be because coffee has strong antioxidant activity with a high capacity for scavenging superoxide radicals (Kitzberger et al., 2014; Sánchez-González et al., 2005) and may improve the defence system against different stressors including heavy metals pollution. Further, caffeine (the main component of RCP) could bind and maintain interactions with some divalent ions through bonds with O and N atoms (Farias, Silva, & Silva, 2003; Nafisi, Sadjadi, Zadeh, & Damerchelli, 2003). In this regard, Lacorte et al. (2013) found that caffeine reduced the cadmium concentration in all tissues analysed. Also, Abdel-Tawwab et al. (2015) fed common carp on RCP-enriched diets for 10 weeks and further exposed to 5.0 mg Zn/L for 7 days and they found that RCP supplementation could reduce Zn bioaccumulation in fish body. In a similar study with other feed supplements, Abdel-Tawwab (2015b) found that American ginseng supplementation reduced copper toxicity for Nile tilapia. Abdel-Tawwab, Mousa, and Abbass (2007) investigated the protective effect of organic selenium (OSe) supplementation from copper toxicity on African catfish, *Clarias gariepinus*. They found that the supplementation of 0.3 g OSe/kg diet could reduce significantly copper residue in fish body. Likewise, Abdel-Tawwab and Wafeek (2010) evaluated the OSe co-administration with waterborne cadmium toxicity for Nile tilapia. They concluded that the supplementation of 0.5 g OSe/kg diet reduced the harmful effect of waterborne cadmium toxicity on fish and reduced significantly cadmium residues in fish body.

5 | CONCLUSION

It could be concluded from the present study that the inclusion of RCP in practical diets for common carp could not improve fish growth and feed utilization. The RCP supplementation also sustained antioxidant enzymes activities and its co-administration with Zn could exert a protective effect against Zn toxicity reducing Zn bioaccumulation in fish body.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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