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Impact of dietary curcumin administration along with feed-born silver nanoparticle on growth, hemato-biochemical parameters, and digestive enzyme activity of common carp (*Cyprinus carpio*)

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Abstract

This research explored the impacts of feed-born silver nanoparticles (AgNPs) on common carp (*Cyprinus carpio*) and whether dietary curcumin supplementation could ameliorate the impacts of AgNPs on growth, hemato-biochemical parameters and digestive enzyme activity. Nine experimental diets were prepared containing 0.00, 0.05, and 0.15 g kg^{-1} AgNPs, as well as 0.00, 0.75, and 1.50 g kg^{-1} curcumin in a factorial design. Triplicate groups of common carp (4.82 ± 0.41 g) were fed on the test diets for 60 days. The results demonstrated that AgNPs reduced growth performance and enhanced the feed conversion ratio dose-dependently. Supplementing 0.75 g kg^{-1} curcumin at a low AgNP level improved the growth rate, while its inclusion at a high AgNP level led to further suppression of growth performance. The highest hematocrit value, hemoglobin concentration and white blood cell count were recorded in the group receiving 0.75 g kg^{-1} curcumin. Serum glucose, cholesterol and triglyceride concentrations were elevated by increasing AgNP levels. However, curcumin inclusion, particularly at the lower level of AgNPs significantly decreased their values. Similarly, intestinal alkaline protease and lipase activities were progressively reduced by increasing dietary AgNP contents, but, significant improvements were observed by curcumin application at the lower AgNP level. Our results revealed that curcumin supplementation could limit the toxic effects of lower dietary AgNP contents.

Introduction

The rapid development of nanotechnology is coupled with environmental hazards from their discharge to water bodies.[1] Once discharged into the environment, nano-particles (NPs) would involve various environmental processes from soil to water with subsequent unclear impacts on the microbial communities of fish and mammals. It has been estimated that ca. 35.00% of nano-silver (Ag) products can potentially release Ag or silver nanoparticles (AgNPs) to the environment.[2] Simulated landfill experiments represented that the remaining Ag particles on the disposed textile were likely to leach into the ambient environment.[3] The environmentally relevant concentration of Ag in water bodies can range from 0.03 to 500 ng L^{-1} with toxic concentrations greater than 50.00 ng L^{-1}. In addition, it has been stated that the Ag concentration might increase 50 folds in coastal waters. Furthermore, the Ag content of sediments was estimated to be 10,000 times higher than the surrounding water. In the sediments, the element is likely to bind organic matters and transfer to benthic detritivores animals including common carp (*Cyprinus carpio*). It was also reported that phytoplankton can bio-concentrating Ag up to 10,000 - 70,000 times more than that possibly found in the water column making Ag the second-rank element after mercury regarding its uptake from the ambient water in comparison with other heavy metals such as zinc, cadmium and copper (Cu).[2]

Various factors including the ion chemical form and water chemistry might affect Ag toxicity. Meanwhile, it has been postulated that NPs act as Trojan horses to deliver free Ag ions to the level of the cellular membrane which changes the scenario of Ag toxicity and warrants further future research. In aquatic systems, NPs can impact untargeted animals including fish and aquatic birds. In addition, they may cause complex damage at the level of cells, tissues, organs and the organism. Various studies have focused on the probable effects of NPs within a water

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body and their interactions with aquatic animals.[4-6] However, the effects of NPs on physiological responses (e.g., blood indices and digestive tract) mainly through food/feed delivery have been controversial.[7]

The application of antioxidants has gained attention because of their mitigating impacts on the treatment of chronic diseases and the prevention of oxidative stress.[8] Dietary antioxidants can reshape carcinogen activation and detoxifying mechanisms in various animals.[9,10] Curcumin is a phenolic secondary plant metabolite that belongs to the ginger family (*Zingiberaceae*) and has long been used as herbal medicine and dietary supplement.[11] Its antioxidative, anti-inflammatory, anti-mutagenic, anti-carcinogenic and anti-microbial properties have been well established in previous studies.[12-14] Its antibacterial activity has been attributed to its mono-carbonyl and bioactive conjugated derivatives.[15,16] The beneficial impacts of curcumin on hepatic oxidative enzyme activity[17] and gut microbiota[18] have been reported, as well. The toxicity and carcinogenicity of heavy metals were mainly attributed to the overproduction of free radicals leading to oxidative stress. Thus, the dietary inclusion of different antioxidants might provide better protection against the over-production of free radicals and exhaustion of cellular antioxidant capacity.

Phytogenics are commonly regarded as a growth promoter, immune-stimulant and antibacterial agent in the nutrition-based health management approach for animal production. For instance, the dietary curcumin contents of 5.00 - 10.00 g kg^{-1} increased body protein content and inhibited liver lipid peroxidation in climbing perch *Anabas testudineus*.[17] Moreover, the supplementation of 20.00 g kg^{-1} curcumin considerably improved the growth indices and feed conversion ratios (FCR) of the rainbow trout.[19] Additionally, 50.00 or 100 mg kg^{-1} feed inclusion of curcumin could improve growth indices and feed performance (i.e., FCR and protein efficiency ratio of tilapia fish).[20] Similarly, the dietary inclusion of 5.00, 10.00, and 20.00 g kg^{-1} curcumin demonstrated promising results in terms of growth indices and feed efficiency of Nile.[21]

This research assessed the probable influence of feed AgNP contamination on the growth, hemato-biochemical parameters and digestive enzyme activity of common carp. In addition, the protective effects of dietary curcumin supplementation on the potential damage of dietary AgNPs contamination were explored.

Materials and Methods

Experimental diets. The Ag nano-powder (99.99%, 20.00 nm, metal basis) was purchased from US Research Nanomaterial Inc. (Houston, USA).[18] All chemicals, solvents and culture media were of analytical grade and supplied from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, USA). The trial was undertaken including nine treatments consisting of different combinations of dietary AgNPs (0.00, 0.05, and 0.15 g kg^{-1} feed) and curcumin (0.00, 0.75, and 1.50 g kg^{-1} feed) levels. After mixing the dry ingredients oil and distilled water were added and the pellets were produced and dried at 45.00 ˚C overnight.[18] Finally, the pellets were crumbled, sieved to remove feed dust and stored at 4.00 ˚C in plastic bags.

Fish husbandry. The fingerlings of common carp (4.82 ± 0.41 g) were obtained from a local farm. The 675 fingerlings were distributed into 27 fiberglass tanks (150 L water). They were fed 0.50% body weight (BW) once daily with the basal diet to adapt them to the experimental conditions for 14 days. The experiments were conducted under natural photoperiod and the fish were fed with nine test diets three times a day at the rate of 3.00% BW for 60 days. The water pH (7.30), dissolved oxygen content (7.60 mg L^{-1}) and temperature (23.00 ˚C) of the tanks were monitored daily. In addition, the daily water exchange rate was 50.00%. The experimental fish care and handling procedures were approved by the Urmia University Animal Ethic Committee, Urmia, Iran (IR-UU-AEC-3/32).

Sample collection. Before sampling, the fish were fasted for 24 hr and anesthetized with a 200-ppm clove oil solution to minimize stress on the fish. Fish number and mass weight in each tank were determined to estimate growth parameters[22] and survival rates. Six fish from each treatment were randomly taken and anesthetized and blood was withdrawn from the caudal vein using 2.00 mL sterilized hypodermic syringes and divided into two portions. The first portion was placed in heparin-coated test tubes and used for hematological parameters analyses. The second portion was allowed to clot at room temperature for 30 min and centrifuged at 1,500 *g* for 15 min and the serum was separated and kept at –80.00 ˚C for biochemical parameters analyses.

Hematological parameters. Red blood cell (RBC) and white blood cell (WBC) counts were quantified with a Neubauer hemocytometer. Also, hematocrit (Ht) and hemoglobin (Hb) concentration were measured using microhematocrit and the cyanomethemoglobin methods, respectively. Differential leukocyte counts were determined using the Giemsa staining method under a light microscope (BH2; Olympus, Tokyo, Japan).[23] Corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated as described by Imani *et al*.[23]

Blood biochemical parameters. Albumin, glucose, cholesterol, and triglyceride concentrations were analyzed by commercial kits (Pars Azmoon, Tehran, Iran) using a microplate reader (HT; Biotek Synergy, Winooski, USA). The serum total protein level was determined by a protein assay kit (Pars Azmoon) using bovine serum albumin as the standard. The activity of inflammatory enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was quantified based on the

International Federation of Clinical Chemistry and Laboratory Medicine. Moreover, the other activities such as alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were quantified according to the Deutsche Gesellschaft fur Klinische Chemie (DGKC) method and in compliance with DGKC (P-L), respectively.[24]

Digestive enzyme activity. To assay digestive enzyme activity, six fish were randomly taken from each treatment and anesthetized as mentioned previously. Then, they were humanely sacrificed by a sharp blow to the head. After removing visceral fat on ice, the intestine was dissected, rinsed with cold 0.90% NaCl solution and kept at –80.00 ˚C until extraction. The samples were homogenized in 1:3 (w v^{-1}) cold 50.00 mM Tris-HCl buffer (pH = 7.50) using Polytron PT 1300 D homogenizer (Metrohm AG, Herisau, Switzerland) with a 7.00 mm generator at a setting of 10 for 3 × 30 sec. The homogenate was centrifuged at 10,000 *g* for 20 min at 4.00 ˚C and the supernatant was separated and kept at – 80.00 ˚C until analyses.[25] Amylase activity was measured through a starch-iodine detection method described by Métais and Bieth.[26] Briefly, 50.00 µL of the enzymatic extract was mixed with the substrate (3.00 g L^{-1} starch in 66.00 mM Na_2PO_4) and left for 20 min at 25.00 ˚C. The reaction was halted with 20.00 µL of 1.00 N HCl, and after the addition of 2.00 mL of 0.33 mM iodine solution, the absorbance was read by the microplate reader at 580 nm. One unit of α-amylase activity was defined as the mg starch hydrolyzed per min at 25.00 ˚C. The activity of lipase was measured by the hydrolysis of nitrophenyl myristate. Each assay (0.50 mL) contained 0.53 mM nitrophenyl myristate, 0.25 mM 2-methoxy ethanol, 5.00 mM sodium cholate, and 0.25 M Tris-HCl (pH = 9.00). Incubation was performed for 15 min at 30.00 ˚C, and the reaction was stopped by adding 0.70 mL of acetone/n-heptane (5/2, v/v). The reaction mixture was vigorously mixed and centrifuged at 6,080 *g* for 2 min. The absorbance of the resulting lower aqueous layer was measured by the microplate reader at 405 nm. The extinction coefficient of n-nitrophenol was 16,500 M^{-1} cm^{-1} per L. One unit of the enzyme activity was defined as 1.00 µmol of n-nitrophenol released per min.[27] Alkaline protease activity was measured as described by Chong *et al*.[25] using 2.00% Azocasein in Tris-HCl (pH = 7.50) as a substrate. The specific enzyme activity was reported as unit activity mg^{-1} protein per min.

Statistical analysis. All statistical analyses were performed using IBM SPSS (version 20.0; IBM Corp., Armonk, USA). The homoscedasticity of the variance of the dependent variables was checked by Levene's test. The standard normality test of Kolmogorov-Smirnov was applied to determine the normality of the data set. Two-way ANOVA was used to determine whether or not there were significant differences among various experimental groups. Tukey's HSD test at $p < 0.05$ was employed to assess significant differences among treatments.

Results

Growth performance. The two-way ANOVA results revealed that all growth indices were affected by two-way interaction of dietary AgNPs and curcumin supplementation ($p < 0.05$, Table 1). Dietary inclusion of AgNPs led to a significant reduction ($p < 0.05$) in growth performance dose-dependently. Curcumin supplementation in diets containing 0.05 g kg^{-1} AgNPs improved growth performance, however, its inclusion at a higher level of AgNPs aggravated growth depression. A progressive increase in the FCR was observed by increasing the AgNP level, and supplementing 0.75 g kg^{-1} curcumin at a lower level of AgNPs decreased this ratio. A similar trend was observed for the fish survival rate. Curcumin supplementation did not influence the above-mentioned parameters compared to the control group.

Hemato-biochemical parameters. All hemato-biochemical parameters were interactively affected by dietary AgNPs and curcumin contents ($p < 0.05$). The Ht and Hb concentrations, WBC, and RBC counts were significantly decreased by the AgNP level and curcumin inclusion in diets containing 0.05 g kg^{-1} AgNPs significantly enhanced their values (Table 2). Moreover, the highest Ht, Hb, WBC, and RBC values were detected in fish fed a diet containing 0.75 g kg^{-1} curcumin. Higher MCV values were found in groups that received AgNP-containing diets and curcumin supplementation reduced its value. The MCHC was remarkably decreased by AgNP inclusion and a significant improvement was observed by supplementing 0.75 g kg^{-1} curcumin in the diet containing a lower level of AgNPs. Serum glucose, cholesterol and triglyceride concentrations as well as AST, ALT and LDH activities were notably increased by AgNP inclusion and curcumin supplementation at lower AgNP levels decreased their values. The serum protein content was significantly decreased at both levels of AgNPs and the inclusion of Curcumin in diets containing 0.05 g kg^{-1} AgNPs significantly improved the protein concentration. The significant reduction in the ALP activity at the lower level of AgNPs was retrieved by the curcumin application (Table 3).

Digestive enzyme activity. Digestive enzyme activity of common carp fed on the experimental diets was affected by the two-way interaction of dietary AgNPs and curcumin inclusion (Table 4, $p < 0.05$). A dose-dependent decrease was observed in alkaline protease and lipase activities by AgNPs ($p < 0.05$). Curcumin application at a low concentration of AgNPs improved protease and lipase activities, while its inclusion at a high AgNP level caused further reductions in both enzymes' activities. Furthermore, the sole addition of curcumin at both levels reduced lipase activity. The dietary inclusion of AgNPs led to a remarkable increase in the α-amylase activity, while curcumin addition at both AgNP levels decreased the enzyme activity.

Table 1. Growth performance, feed utilization and survival of common carp fed on the experimental diets for 60 days.

Treatment	FBW (g)	WG (g)	SGR (%)	FCR	Survival rate (%)
Control	21.10 ± 0.13^{a}	16.40 ± 0.45^{a}	2.49 ± 0.12^{ab}	2.26 ± 0.10^{f}	98.70 ± 2.31^{a}
Cur0.75	21.10 ± 0.26^{a}	16.30 ± 0.30^{a}	2.48 ± 0.13^{ab}	2.31 ± 0.10^{f}	98.70 ± 2.31^{a}
Cur1.50	21.00 ± 0.18^{a}	16.50 ± 0.12^{a}	2.56 ± 0.08^{a}	2.34 ± 0.10^{f}	90.70 ± 2.31^{a}
AgNPs0.05	16.50 ± 0.49^{c}	11.60 ± 0.60^{c}	2.04 ± 0.16^{cd}	3.04 ± 0.15^{d}	61.30 ± 6.11^{c}
AgNPs0.15	15.50 ± 0.18^{d}	10.60 ± 1.80^{c}	1.93 ± 0.13^{cde}	3.32 ± 0.10^{c}	45.30 ± 2.31^{d}
Cur0.75/AgNPs0.05	17.50 ± 0.23^{b}	12.70 ± 0.53^{b}	2.16 ± 0.07^{bc}	2.73 ± 0.05^{e}	77.30 ± 2.31^{b}
Cur0.75/AgNPs0.15	13.50 ± 0.10^{e}	8.77 ± 0.35^{d}	1.75 ± 0.12^{de}	3.71 ± 0.10^{b}	24.00 ± 4.01^{e}
Cur1.50/AgNPs0.05	16.10 ± 0.11^{c}	11.50 ± 0.40^{c}	2.09 ± 0.12^{cd}	3.01 ± 0.10^{d}	62.70 ± 2.31^{c}
Cur1.50/AgNPs0.15	12.00 ± 0.14^{f}	7.36 ± 0.35^{e}	1.58 ± 0.15^{e}	4.34 ± 0.10^{a}	20.00 ± 4.01^{e}

Cur: Curcumin, AgNPs: Silver nanoparticles, FBW: Final body weight (g), WG: Weight gain = (Final mean body weight – initial mean body weight), SGR: Specific growth rate = [(Ln final body weight − Ln initial body weight) duration (days)] × 100, and FCR: Feed conversion ratio = dry feed fed per wet weight gain, (Ln = natural logarithm).
Values in the same column having different superscripts are significantly different ($p < 0.05$).

Table 2. Hematological parameters of common carp fed on the experimental diets for 60 days.

Treatment	HCT (%)	Hb (g L^{-1})	MCV (fL)	MCHC (%)	WBC (×10^{3} mL^{-1})	RBC (×10^{6} mL^{-1})
Control	25.47 ± 1.16^{a}	5.80 ± 0.30^{ab}	210.75 ± 15.30^{bcd}	22.77 ± 0.20^{ab}	4.77 ± 0.35^{b}	1.21 ± 0.04^{b}
Cur0.75	26.43 ± 1.07^{a}	6.23 ± 0.30^{a}	183.71 ± 10.500^{d}	23.64 ± 2.10^{ab}	6.23 ± 0.31^{a}	1.43 ± 0.04^{a}
Cur1.50	25.13 ± 0.80^{ab}	5.73 ± 0.31^{ab}	183.51 ± 7.49^{d}	22.84 ± 1.70^{ab}	4.87 ± 0.31^{b}	1.37 ± 0.02^{a}
AgNPs0.05	20.77 ± 1.11^{c}	3.73 ± 0.20^{cd}	241.49 ± 11.40^{ab}	18.03 ± 1.70^{d}	3.47 ± 0.25^{cd}	0.86 ± 0.02^{d}
AgNPs0.15	18.23 ± 0.61^{d}	3.43 ± 0.21^{d}	244.16 ± 4.00^{a}	18.83 ± 1.10^{cd}	3.23 ± 0.15^{cd}	0.74 ± 0.02^{e}
Cur0.75/AgNPs0.05	22.87 ± 1.07^{bc}	5.63 ± 0.20^{ab}	183.40 ± 6.10^{d}	24.68 ± 1.80^{a}	4.63 ± 0.15^{b}	1.24 ± 0.03^{b}
Cur0.75/AgNPs0.15	21.27 ± 0.61^{c}	4.27 ± 0.20^{cd}	200.91 ± 12.40^{cd}	20.06 ± 0.10^{bcd}	3.53 ± 0.28^{cd}	1.06 ± 0.04^{c}
Cur1.50/AgNPs0.05	21.27 ± 0.35^{c}	4.84 ± 0.31^{bc}	217.29 ± 11.40^{abc}	22.72 ± 1.00^{ab}	3.77 ± 0.15^{c}	0.98 ± 0.04^{c}
Cur1.50/AgNPs0.15	17.17 ± 0.71^{d}	3.83 ± 0.20^{cd}	197.59 ± 13.90^{cd}	22.34 ± 0.90^{abc}	3.07 ± 0.15^{d}	0.87 ± 0.03^{d}

Cur: Curcumin, AgNPs: Silver nanoparticles, HCT: Hematocrit, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cells count, and RBC: Red blood cells count.
^{abcd} Values in the same column having different superscripts are significantly different ($p < 0.05$).

Table 3. Blood biochemical parameters of common carp fed on the experimental diets for 60 days.

Treatment	Protein (g dL^{-1})	Albumin (g dL^{-1})	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	TG (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})	LDH (IU L^{-1})
Control	3.58±0.23^{a}	0.59±0.06^{ab}	77.33±1.60^{bc}	66.67±0.80^{c}	97.83±1.50^{d}	88.83±1.40^{fg}	21.87±1.30^{d}	74.47±1.41^{a}	218.37±4.10^{ef}
Cur0.75	3.54±0.02^{a}	0.60±0.05^{a}	74.33±1.61^{c}	56.33±1.31^{d}	86.93±1.31^{e}	83.77±1.51^{gh}	16.57±0.71^{e}	77.33±1.02^{a}	208.90±3.11^{f}
Cur1.50	3.46±0.11^{ab}	0.46±0.09^{abc}	60.43±0.90^{d}	53.67±0.80^{d}	79.23±1.40^{f}	82.87±1.81^{h}	17.53±0.71^{e}	77.97±1.01^{a}	214.77±5.60^{ef}
AgNPs0.05	2.83±0.09^{cd}	0.44±0.09^{abc}	82.33±1.71^{a}	83.67±1.12^{a}	116.63±1.20^{b}	134.37±1.70^{d}	39.57±2.00^{b}	48.93±1.80^{b}	269.11±5.21^{c}
AgNPs0.15	2.57±0.13^{d}	0.45±0.10^{abc}	83.57±2.21^{a}	85.33±0.60^{a}	123.67±2.11^{a}	203.73±1.31^{a}	61.43±1.82^{a}	44.37±0.81^{b}	306.63±4.62^{a}
Cur0.75/AgNPs0.05	3.54±0.11^{a}	0.61±0.06^{a}	73.33±1.52^{c}	67.01±1.01^{c}	100.13±1.41^{d}	91.30±1.60^{f}	22.57±1.01^{d}	76.33±2.01 ^{a}	225.83±2.90^{e}
Cur0.75/AgNPs0.15	2.64±0.15^{d}	0.39±0.07^{bc}	53.33±1.40^{e}	48.67±0.61^{e}	72.67±2.10^{g}	186.47±2.81^{b}	62.17±1.31^{a}	34.13±1.37^{c}	288.53±3.50^{b}
Cur1.50/AgNPs0.05	3.14±0.11^{bc}	0.39±0.07^{bc}	80.33±1.50^{ab}	73.01±0.91^{b}	111.87±2.30^{c}	101.93±1.92^{e}	27.97±2.51^{c}	78.47±1.20^{a}	243.03±4.21^{d}
Cur1.50/AgNPs0.15	2.61±0.07^{d}	0.38±0.03^{c}	54.01±1.30^{e}	45.33±1.80^{f}	63.07±1.91^{h}	177.63±1.41^{c}	61.46±1.10^{a}	38.13±1.38^{c}	312.46±4.34^{a}

Cur: Curcumin, AgNPs: Silver nanoparticles, TG: Triglyceride, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, and LDH: Lactate dehydrogenase.
$^{a-h}$ Values in the same column having different superscripts are significantly different ($p < 0.05$).

Table 4. Digestive enzymes activity in common carp fed on the experimental diets for 60 days.

Treatment	Protease (U mg^{-1} protein)	Lipase (U mg^{-1} protein)	Amylase (U mg^{-1} protein)
Control	86.66 ± 2.18^{ab}	43.73 ± 2.04^{a}	35.86 ± 1.22^{cd}
Cur0.75	82.91 ± 2.20^{ab}	37.10 ± 1.41^{c}	31.20 ± 0.91^{e}
Cur1.50	85.66 ± 2.73^{ab}	34.73 ± 2.03^{c}	36.81 ± 1.50^{cd}
AgNPs0.05	59.93 ± 2.43^{d}	37.91 ± 1.35^{bc}	44.53 ± 1.25^{b}
AgNPs0.15	50.83 ± 2.18^{cd}	35.73 ± 1.68^{c}	49.76 ± 1.38^{a}
Cur0.75/AgNPs0.05	86.90 ± 1.93^{a}	44.63 ± 2.21^{a}	33.36 ± 1.49^{de}
Cur0.75/AgNPs0.15	35.81 ± 2.40^{bcd}	23.31 ± 1.90^{d}	21.36 ±1.11^{f}
Cur1.50/AgNPs0.05	80.23 ± 2.11^{ab}	42.63 ± 1.91^{ab}	38.66 ± 1.78^{c}
Cur1.50/AgNPs0.15	38.81 ± 2.25^{abc}	25.26 ± 1.28^{d}	27.11 ± 1.17^{g}

Cur: Curcumin, and AgNPs: Silver nanoparticles.
$^{a-f}$ Values in the same column having different superscripts are significantly different ($p < 0.05$).

Discussion

The discharge of pollutants including nano-sized materials into water bodies leads to their uptake and accumulation in the tissues of aquatic animals subsequently causing gastrointestinal toxicity.[28] Our results revealed that feed-born AgNPs hampered the growth performance of common carp and curcumin supplementation at a low inclusion level could partially prevented the suppressive effect of AgNPs. Moreover, no adverse effect of curcumin was found on fish growth performance which was consistent with the findings of Lee *et al*. and Imani *et al*., indicating no impacts of plant extracts on feed consumption, biomass gain and feed utilization.[29,30] A dose-dependent reduction of the growth performance by AgNPs in this study was in agreement with the depression of growth performance in *Clarias batrachus*[31] and *Epinephelus coioides*[32] by AgNPs and Cu NPs, respectively. However, Ramsden *et al*. reported that the effect of titanium dioxide NPs was null on the growth of the rainbow trout (*Oncorhynchus mykiss*).[33] However, subtle biochemical disturbances were found in the brain.

All hematological parameters except for hematocrit were beneficially influenced by the application of curcumin in AgNP-containing diets. This was in agreement with results obtained later in Ag carp (*Hypophthalmichthys molitrix*)[34] and fathead minnow (*Pimephales promelas*).[35] The reduction of the RBC count and Hb and Ht levels by AgNPs in this study could be due to several reasons such as erythropoiesis disorder and the formation of RBC, the conditions of confinement or stress induced by the lack of food and lysing of RBC as a result of toxicant stress.[36]

Blood parameters, biochemical and hematology, are helpful diagnostic tools. For instance, ALT, AST and LDH are used to recognize disease and tissue damage induced by environmental pollutants. As hepatocytes are damaged, ALT and AST are discharged into the bloodstream.[37] Our results confirmed increased AST, ALT and LDH activities in fish-fed AgNP-containing diets probably indicating cellular membrane damage and increased enzyme leakage which was in agreement with reports on the rainbow trout[23] and Caspian kutum (*Rutilus kutum*).[38] Kumar *et al*. reported the highest Ag content in the liver tissue of fish feeding a diet containing 1.00 mg AgNPs kg^{-1} which was synchronized with higher LDH, AST, and ALT activities in the liver tissue of the group.[39] Accordingly, Kumar *et al*. concluded that increased AST and ALT might be attributable to tissue damage as reflected by the liver histopathology observations representing the marked signs of damage by being fed with 1.00 mg AgNP kg^{-1} diet.[40] Furthermore, our results demonstrated that curcumin inclusion in AgNP-containing diets could remarkably reduce serum AST, ALT and LDH activities implying that curcumin supplementation could protect hepatocytes from AgNP-induced damage. The protective effects of curcumin on blood biochemical parameters could be due to various antioxidative compounds which beneficially influence cellular physiological functions (e.g., improving antioxidant capacity and membrane stability and preventing the leakage of the intracellular enzyme into the blood during oxidative stress).[5]

It has been demonstrated that digestive enzyme activity is responsive to environmental factors and their activity could be regained after eliminating obstacles.[41] Our results revealed that AgNPs suppressed lipase and alkaline protease activities and increased amylase activity. Likewise, Le Bihan *et al*. represented that Ag exposure led to decreased protease activity of cuttlefish (*Sepia officinalis*).[42] Likewise, the results of Wang *et al*. indicated that Cu NPs and Cu sulfate contamination led to decreased digestive enzyme activities in grouper (*E. coioides*).[32] In contrast, Samanta *et al*. reported the enhancement of digestive enzyme activity in *Anabas testudineus*, *Heteropneustes fossilis*, and *Oreochromis niloticus* following herbicide Almix® exposure.[43] These researchers suggested that fish might respond to increased energy requirements by regulating their digestive enzyme profile. However, such increased activity of digestive enzymes could also indicate pancreatitis incidence,[44] which should be clarified in the future. Our data showed that the sole curcumin supplementation reduced lipase and amylase activities compared to the control. Furthermore, its inclusion in diets containing 0.05 g kg^{-1} AgNPs retrieved reduced lipase and protease activities. Similarly, Imani *et al*.[30] found that cinnamon oil supplementation in diets for rainbow trout lowered digestive enzyme activity. Similarly, Nazdar *et al*.[45] concluded that the application of silymarin in rainbow trout feed could partially mitigate NiO-NP toxicity. However, in this study, curcumin application failed to protect the fish at higher AgNP doses.

In conclusion, our results suggested that curcumin supplementation in the diet containing Ag-NP 0.05 g kg^{-1} could retrieve the adverse effects of NPs on different studied parameters including growth and nutritional indices, serum biochemistry and digestive enzyme activity.

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Conflict of interest

The authors declare no conflicts of interest.

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