



# Dietary supplementation with curcumin nanomicelles, curcumin, and turmeric affects growth performance and silver nanoparticle toxicity in *Cyprinus carpio*

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## Abstract

An 8-week feeding experiment was conducted to investigate the effects of curcumin nanomicelle, curcumin, and turmeric (*Curcuma longa*) on growth performances, body composition, fatty acid profile, and biochemical parameters of common carp (*Cyprinus carpio*), and their ameliorative effects against toxicity of silver nanoparticles (AgNPs). A total of 120 healthy carps were randomly distributed into four equal treatments. Curcumin nanomicelle, curcumin, and turmeric were each added separately to the basal diet. After the feeding trials, all treatments were exposed to a non-lethal concentration of AgNPs ( $0.5 \text{ mg L}^{-1}$ ) for 96 h. Fish fed dietary turmeric showed a significantly higher weight gain. The body protein content was significantly increased in all feeding groups, while the lipid content showed a significant decrease in the turmeric-treated group. Dietary turmeric improved the concentration of saturated fatty acids (SFA) and monounsaturated fatty acid (MUFA). AgNP exposure led to increases in liver catalase (CAT) activity of carps fed with turmeric and curcumin. The lowest amount of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was obtained in fish fed with nanomicelle curcumin and curcumin diets. The lowest amount of silver accumulation in the liver of carps was found in fish fed with dietary curcumin nanomicelle. This experiment suggests that supplementation of turmeric ( $50 \text{ g kg}^{-1}$ ) or curcumin ( $1000 \text{ mg kg}^{-1}$ ) may play an important role in enhancing growth performances and fatty acid composition of the common carp. Moreover, administration of curcumin nanomicelle in the diet may have a potential ameliorative effect against toxicity of AgNPs.

**Keywords** Bioaccumulation · Common carp · Fatty acids · Nanoencapsulation · Nanotoxicity

## Introduction

Nanotechnology is being used to produce different products in the global market, some of which are based on silver

nanoparticles (AgNPs). The unique properties of AgNPs, including surface functionalization, chemical stability, and catalytic activity (Buzea et al. 2007; Krutyakov et al. 2008), make them valuable materials for different industries such as cosmetics, medicine, household, etc. (De et al. 2008; Raj et al. 2012). However, widespread use of AgNPs is not free from undesirable side effects (Yu et al. 2013). Nowadays, many experimental studies have been conducted to evaluate the negative consequences of releasing nanoresidues into the aquatic ecosystems (Asadi Dokht Lish et al. 2019; Haghghat et al. 2021; Zeumer et al. 2020). In nano-ecotoxicological studies, focus was set on the bioavailability and bioaccumulation of AgNPs at the whole-organism level, followed by the route of absorption and physiological changes (McGillicuddy et al. 2017). It has been shown that the toxicity of AgNPs is dependent on their particle sizes (Ivask et al. 2014). In rainbow trout (*Oncorhynchus mykiss*) exposed to different forms of AgNPs, smaller sizes of AgNPs were detected in the liver, while larger sizes were mainly accumulated in the intestine (Johari et al. 2015). Also, Joo et al. (2018) showed

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that the vulnerability of rainbow trout to AgNPs is concentration-dependent, irrespective of water salinity.

Encapsulation of nutrients using nanotechnology is another aspect that has received much attention. Availability and accessibility of nutrients would be elevated using this method by loading a nutrient (Moniruzzaman and Min 2020; Veisi et al. 2021). Nanomicelles are one of the nanocarriers that consist of an external hydrophilic region and an internal hydrophobic core (Vaishya et al. 2014) that has been used in drug delivery systems to improve permeability and drug bioavailability (Vadlapudi and Mitra 2013).

Two grand overarching strategies of aquaculture are increasing the growth rate and improving the meat quality (Baldissera et al. 2020). In this regard, previous findings have shown that medicinal plants as feed additives in the diet not only minimize the production cost, but also reduce negative side effects of synthetic drugs in both fish and environment (Flück and Jaspersen-Schib 1976). Turmeric, *Curcuma longa*, that belongs to the Zingiberaceae family possesses unique properties such as anti-inflammatory (Nonose et al. 2014), antimicrobial (Abdel-Tawwab and Abbass 2017; Sahu et al. 2008), antioxidant (Manju et al. 2012; Rajabiesterabadi et al. 2020), and immunomodulatory (Abdelrazek et al. 2017; Sahu et al. 2008). Curcumin, which is a polyphenol obtained from *C. longa*, is the main biological active component found in turmeric and has a wide range of physiological activities such as antistress (Akdemir et al. 2017) and immunostimulant (Kohshahi et al. 2019). Curcumin nanomicelles, hereafter called as “curcumin-NM,” has been recently developed as a new nano-based structure to enhance physicochemical properties of curcumin such as stability and solubility (Li et al. 2017). Considering the importance of turmeric and curcumin on the immune system quality, it is important to address whether curcumin-NM supplementation may influence the growth performances and biochemical properties. As far as the authors are aware, the impact of dietary curcumin-NM on fish species has not been investigated; therefore, curcumin-NM as the novel form of curcumin was used as a feed additive in the diet of common carp to assess its nutritional benefits.

The common carp, *Cyprinus carpio*, is one of the major freshwater species produced in the world that in 2016 it ranked third in terms of worldwide finfish production (FAO 2018). Taken together, the present study was carried out to investigate the effects of curcumin-NM, curcumin, and turmeric on the growth performances, body composition, and fatty acid profile, and also to determine ameliorative effects of experimental diets on common carp exposed to AgNPs.

## Materials and methods

### Preparation of experimental diet

The commercial feed for carps was purchased (Dan-e-Rose. Co., Iran) and had the following proximate composition:

protein ( $36.0 \pm 2.2$ ), lipid ( $9.5 \pm 1.4$ ), ash ( $9.0 \pm 1.2$ ), and moisture ( $10.1 \pm 1.3$ ). Curcumin nanomicelles (SinaCurcumin®,  $9.3 \pm 0.2$  nm) were provided by Exir Nano Sina company (transmission electron microscopy, TEM; images are also shown in Fig. 1a), Tehran, Iran, and contained 6.65% curcuminoids (72% curcumin, 25% desmethoxycurcumin, and 3% bisdemethoxycurcumin). The curcumin powder ( $C_{21}H_{20}O_6$ ,  $\geq 75.0\%$  curcumin,  $\leq 5.0\%$  bisdemethoxycurcumin,  $\leq 20.0\%$  demethoxycurcumin) was purchased from Merck and freshly powdered turmeric (contained 2% curcumin) was purchased from a local market. First, the food was completely powdered by mill and then freshly powdered turmeric ( $50,000$  mg  $kg^{-1}$ , equivalent to  $1000$  mg  $kg^{-1}$  curcumin), curcumin powder ( $1000$  mg  $kg^{-1}$ ), and curcumin-NM ( $15$  mL  $kg^{-1}$ , equivalent to  $1000$  mg  $kg^{-1}$  curcumin) were added and mixed to the experimental basal diet. Experimental diets then were dried at  $40$  °C for 24 h and stored in  $-20$  °C until use. The control group was fed with basal diet. The level of incorporated turmeric to the diet was chosen according to the intermediated amount reported by (Ferreira et al. 2017).

A total of 120 healthy carps with the initial average weight of  $26.32 \pm 4.37$  g (mean  $\pm$  SD) were purchased from a local hatchery (Qasr-e Shirin, Kermanshah, Iran) and were acclimated to laboratory conditions (Aquatic Nanobiotechnology Lab, University of Kurdistan, Sanandaj, Iran) for 1 week; during that time, they were kept in 500-L tanks and fed on basal diet. After that, the fish were randomly distributed to 12 glass aquaria (150 L), resulting in ten fish per aquarium, and they were acclimated for one more week before the feeding trial in aquaria. During the experiment, the photoperiod, temperature, pH, and dissolved oxygen (DO) were maintained at 16L:8D,  $19.40 \pm 3.08$  °C,  $7.8 \pm 0.2$ , and  $6.9 \pm 0.5$  mg  $L^{-1}$ , respectively. About 70% of water changes were done every day. Fish were fed three times per day at 3% of their body weight (Ashouri et al. 2015) for the first 40 days, and 4% for the next 20 days. No mortality was observed during the feeding experiment.

### Growth performance

At the end of the feeding experiment, fish were anesthetized by MS-222 (tricaine methanesulfonate) and growth performance parameters were calculated by using the following equations:

weight gain (WG, g)

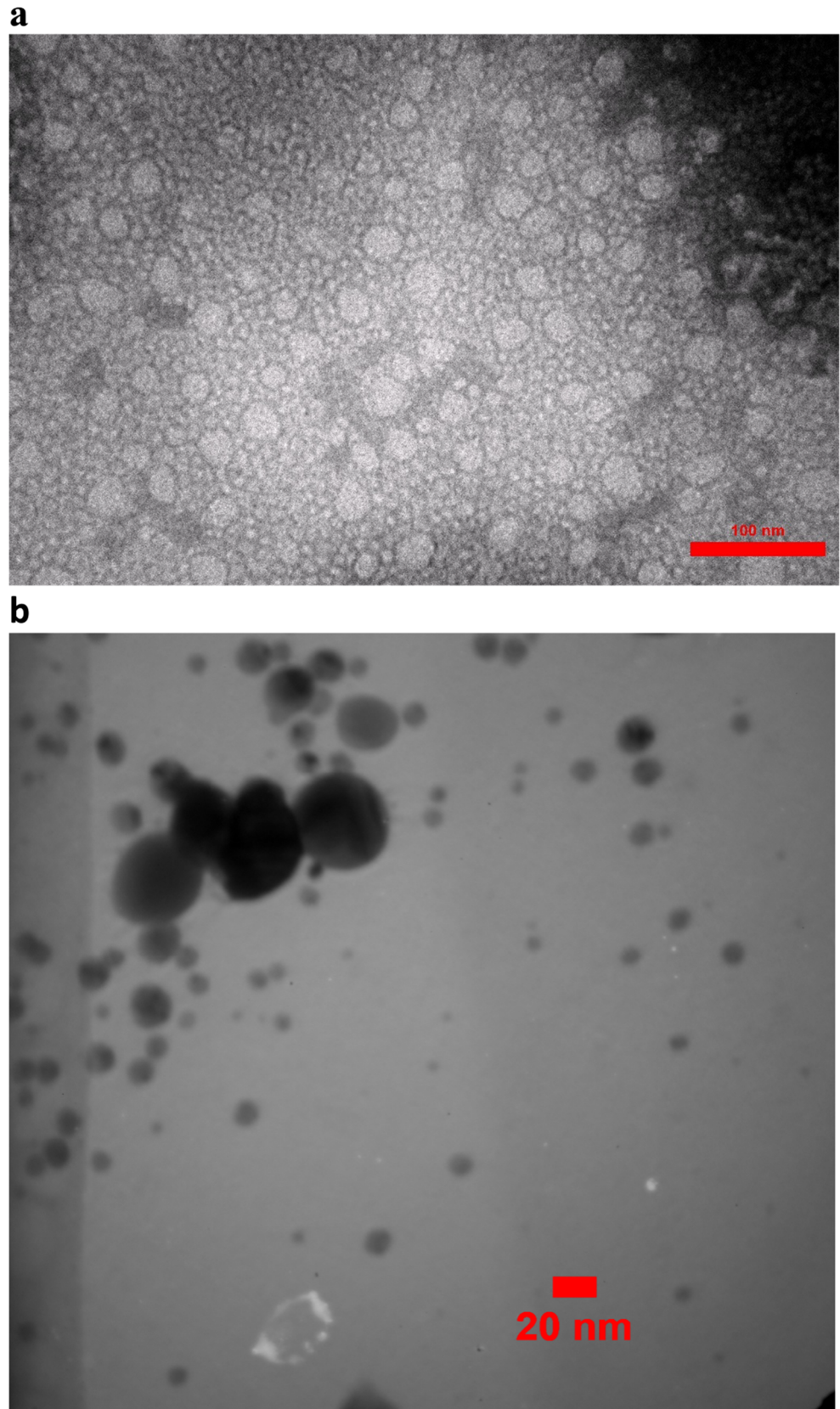
$$= \text{final body weight} - \text{initial body weight}$$

specific growth rate (SGR, %per day)

$$= 100$$

$$\times \frac{(\text{Ln final body weight}) - (\text{Ln initial body weight})}{\text{rearing days}}$$

**Fig. 1** Transmission electron microscope (TEM) images of **a** curcumin nanomicelles and **b** silver nanoparticles (AgNPs)



$$\text{hepatosomatic index (HSI, \%)} = 100 \times \frac{\text{liver weight (g)}}{\text{body weight (g)}}$$

$$\text{condition factor (CF, g cm}^{-3}\text{)} = 100 \times \frac{\text{body weight (g)}}{\text{body length}^3 \text{ (cm}^3\text{)}}$$

### Blood collection

After the feeding trial, blood samples were collected from the caudal vein by using a 1-mL plastic syringe and centrifuged (4000 rpm) for 15 min at 4 °C. The plasma sample then was separated and stored at – 80 °C until use. Anesthetized fish then were sacrificed by rapid pithing, eviscerated, and stored at – 80 °C to measure the proximate composition and fatty acid profile.

### Proximate composition and fatty acid profile

Five fish from each replicate were used to perform proximate composition analysis. Crude protein, lipid, ash, and moisture were determined according to AOAC (1990). Crude protein and crude lipid were measured using the Kjeldahl and Soxhlet methods, respectively. Moisture was determined by oven drying at 105 °C to a constant weight, and ash was determined by combusting the dry samples at 550 °C for 24 h.

To determine fatty acid composition, total lipid was extracted from samples according to Folch et al. (1957). Briefly, tissues (max 1 g) were homogenized in a solvent mixture of chloroform:methanol (2:1) and kept for 24 h. The solvent was removed from samples under nitrogen gas by using a water bath. For sample esterification, 5 mL of sodium hydroxide 2% was added to extracted lipid, vigorously shaken, and kept for 10 min in the water bath. Then, 3 mL of 20% boron trifluoride-methanol (BF<sub>3</sub>) was added to the samples and kept at room temperature for 3 min. The samples were recovered by adding 1 mL hexane and 1 mL of sodium chloride 30% (AOCS 1998). Gas chromatography analysis was conducted using a Philips gas chromatograph equipped with a flame ionization detector (FID) and silica capillary column (30 m × 0.25 mm, ID × 0.22 μm, SGE BPX70). Helium was used as the carrier gas. The temperature was set at 160 °C for 5 min, followed by an increase to 180 °C at a rate of 20 °C/min and then to 200 °C at 1 °C/min and then again to 230 °C at 30 °C/min. The final temperature was maintained for 5 min using the retention time of standard mixtures which allowed the identification of fatty acids.

### Plasma biochemical parameters

The plasma biochemical parameters including glucose (mg dL<sup>-1</sup>), cholesterol (mg dL<sup>-1</sup>), and total protein (g dL<sup>-1</sup>) were measured after the feeding trial using commercial kits (Pars-

Azmun Chemical Company, Tehran, Iran) according to the manufacturer's instructions.

### AgNP exposure experiment

The AgNPs used in this study were from exactly the same stock that was well characterized, and their properties were reported in our recently published work (Tayemeh et al. 2020). In addition, TEM analyses of AgNPs were performed using a Carl Zeiss AG - Zeiss EM900 transmission electron microscope (Fig. 1b). After the feeding trial, the remaining fish (*n* = 5) in each aquarium were distributed to the exposure aquaria. The exposure trial consisted of three experimental groups (fed by curcumin-NM-, curcumin-, and turmeric-supplemented diets and exposed to AgNPs), a positive control group (fed by a basal diet and exposed to AgNPs), and a negative control group (fed by a basal diet and were not exposed to AgNPs). Fifteen aquaria (40 L capacity) were filled with fresh-aerated water up to 30 L. Fish were exposed for 96 h to one non-lethal concentration of AgNPs (0.5 mg L<sup>-1</sup>) which was selected by a series of preliminary range finding tests. At the end of the exposure experiment, all of the fish were anesthetized, blood samples were collected, and plasma were separated and kept at – 80 °C until use. Also, the livers were taken from all of the fish for measurement of silver accumulation and stored at – 80 °C until use.

### Hepatic enzyme activity

For the livers of two individual fish, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured calorimetrically using commercial kits supplied by Pars-Azmun Chemical Company, Tehran, Iran, according to the manufacturer's instructions.

### Antioxidant status

The livers of 3 individual fish for measurement of antioxidant enzyme activity were thawed, and 100 mg of each was homogenized with 1 mL ice-cold phosphate-buffered saline (PBS; pH 7.4) for 2 min. Then, the samples were centrifuged (10,000 rpm) for 15 min at 4 °C and the supernatant was obtained and stored at – 80 °C. Plasma catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) activities were measured using commercial kits (Zellbio, Germany) according to the instructions of the manufacturing company.

### Accumulation of silver in liver

Liver samples were kept at – 20 °C for 48 h, freeze-dried for 24 h, weighed, and digested by concentrated nitric acid. For 0.2 g of sample, 3 mL of concentrated nitric acid (Supelco™)

was added and kept for 1 h in a water bath at 100 °C. After cooling, the samples were filtered through a paper filter and made up to 10 mL volume with ultrapure water. Silver concentration was measured with the Perkin-Elmer pinAAcle 900T atomic absorption spectrophotometer in the Institute of Nanoproduct Safety Research (University of Hoseo, South Korea).

### Statistical analysis

The normal distribution of data was evaluated by the Kolmogorov-Smirnov test. The significant differences between groups were detected by Duncan's multiple range test followed by one-way analysis of variance (ANOVA). The software SPSS version 16 was used for statistical analysis. In all cases, the significance level was set at  $P < 0.05$ .

## Results

### Growth performances

Results obtained for growth performances are shown in Fig. 2. Fish fed 50 g kg<sup>-1</sup> turmeric diet showed a significantly higher WG ( $P < 0.05$ ) in comparison to other experimental groups, but no significant difference was observed between the curcumin and curcumin-NM groups. The mean HSI of the control was significantly higher than that of the other trials except for that of the curcumin group. The values of CF were significantly lower ( $P < 0.05$ ) in the turmeric group than in the other experimental groups. However, no significant difference was observed in SGR among the experimental groups.

### Proximate composition

The effect of different dietary supplements on the body composition of carps after 2 months of feeding is presented in Table 1. The content of protein in fish fed turmeric, curcumin, and curcumin-NM was significantly higher ( $P < 0.05$ ) than that of the control group. Also, the lipid content after the 8-week feeding trial was decreased significantly ( $P < 0.05$ ) by dietary turmeric supplementation. Ash content was significantly increased in fish fed curcumin compared to other experimental groups. No significant difference was observed in moisture content among the treatment groups.

### Fatty acid profile

The fatty acid composition of carps after the 8-week feeding trial is shown in Table 2. Fish fed dietary supplemented with turmeric and curcumin showed a significantly higher concentration of saturated fatty acids ( $P < 0.05$ ). Among the SFAs, palmitic acid (C16:0) and stearic acid (C18:0) contents were

significantly higher in fish fed curcumin. Similarly, the mean concentration of MUFA was markedly higher ( $P < 0.05$ ) in fish fed curcumin followed by turmeric. However, no significant difference was observed in the content of MUFA between the curcumin-NM and control groups. Palmitoleic acid (C16:1) and oleic acid (C18:1(n-9)) were the predominant MUFA in carp muscles fed with curcumin. On the other hand, the lowest level ( $P < 0.05$ ) of PUFA was observed in fish fed diet supplemented with curcumin followed by the turmeric group. The highest concentration of PUFA was found in the control group. The PUFA, EPA, and DHA levels of the group supplemented with 1000 mg kg<sup>-1</sup> curcumin-NM were found to be higher ( $P < 0.05$ ) than those of the other groups.

### Biochemical indices

Biochemical blood parameters after the 8-week feeding trial are shown in Fig. 3. The levels of glucose, cholesterol, and total protein at the end of the 8-week feeding trial showed no significant differences between the experimental groups ( $P > 0.05$ ).

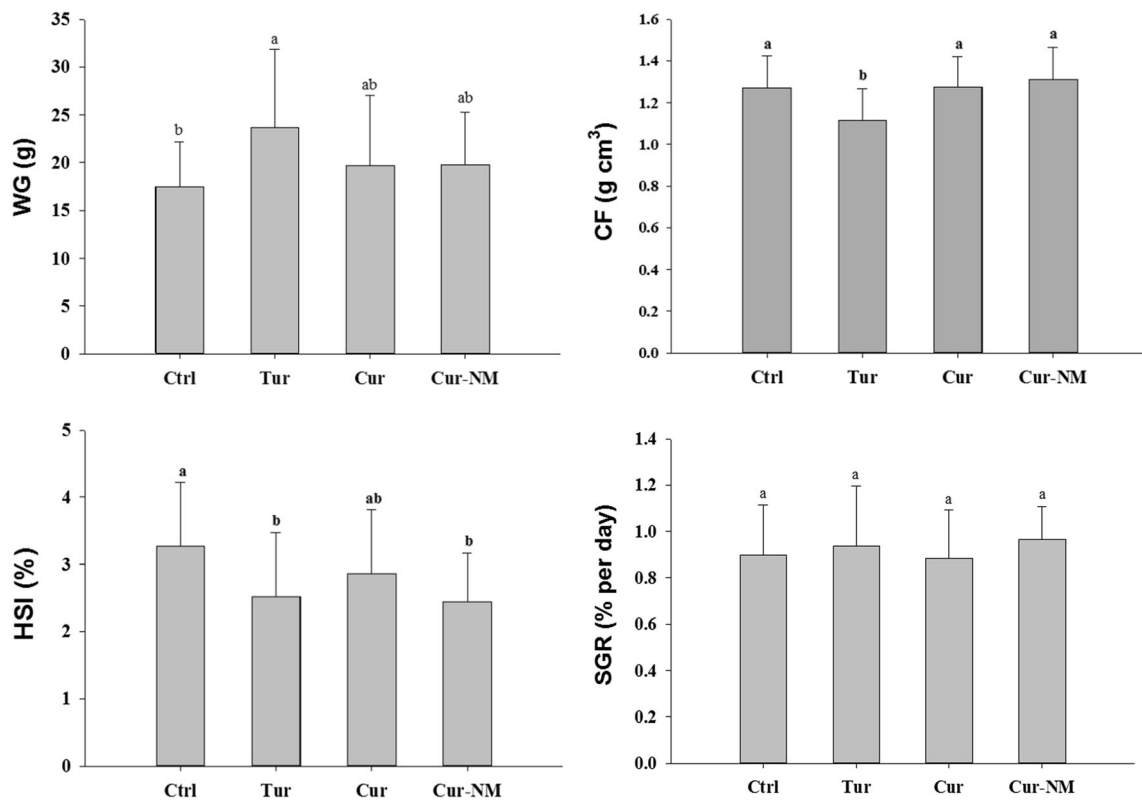
The activities of AST and ALT after the exposure experiment are presented in Fig. 4. After the 96-h nanosilver exposure, the highest and lowest levels of AST were observed in the negative control and curcumin groups, respectively. Similarly, the negative control group showed the highest level of ALT ( $P < 0.05$ ), but no significant differences were observed between ALT levels of the other experimental groups following AgNP exposure.

### Antioxidant status

Antioxidant responses of carps after exposure to 0.5 mg L<sup>-1</sup> AgNPs are shown in Fig. 5. Exposure to AgNPs caused a decrease of the liver CAT in fish that fed on basal diet ( $P < 0.05$ ), but the levels of CAT activity in fish fed dietary turmeric and curcumin were higher than those of the negative control group ( $P < 0.05$ ). On the other hand, the concentration of MDA increased significantly ( $P < 0.05$ ) in the negative control group, but no differences were found among other experimental groups. The liver SOD and GPx activities, however, remained unchanged over the exposure between test groups.

### Silver accumulation in liver

The changes of silver content in the liver of carps after the 96-h exposure are shown in Fig. 6. Exposure to AgNPs caused severe accumulation of silver in the liver tissue of fish fed basal diet and dietary turmeric ( $P < 0.05$ ). Moreover, silver accumulation in the liver of carp fed dietary curcumin was



**Fig. 2** Growth indices of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet (Ctrl), turmeric (Tur), curcumin (Cur), and curcumin nanomicelle (Cur-NM). Bars with different letters are significantly different (mean ± SD, Duncan,  $P < 0.05$ )

higher than that of carp fed curcumin-NM, although statistically it was not significant ( $P > 0.05$ ).

### Discussion

Recently, large number of experiments were conducted to investigate the effects of turmeric and curcumin as feed additives on important farmed fish species. Moreover, the potential effects of dietary nanoencapsulated curcumin have received less attention (Shah and Mraz 2020). Thus, the present study was designed to evaluate the effects of turmeric, curcumin, and nanoencapsulated curcumin individually over an 8-week feeding trial followed by a 96-h nanosilver exposure.

In the present study, fish fed with the 50 g kg<sup>-1</sup> turmeric diet showed a higher rate of WG than the other experimental groups. It has been shown that administration of turmeric up to 5 mg kg<sup>-1</sup> diet could improve the final weight of rohu (*Labeo rohita*) and common carp (Abdel-Tawwab and Abbass 2017; Sahu et al. 2008). In contrast, Abdelrazek et al. (2017) reported a negative effect in the growth performances of Nile tilapia (*Oreochromis niloticus*) fed more than 2 g kg<sup>-1</sup> turmeric diet. Also, no differences were found in juvenile characin, *Astyanax aff. Bimaculatus*, fed with different levels of turmeric (Ferreira et al. 2017). Fish species have responded differently to dietary turmeric that might be related to the species-specific digestive function. However, turmeric has been reported as a potential stimulant for the activity of digestive enzymes (Prasad and Aggarwal 2011).

**Table 1** Body composition of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet, turmeric, curcumin, and curcumin nanomicelle (curcumin-NM)

Treatments	Control	Turmeric	Curcumin	Curcumin-NM
Protein (%)	12.95 ± 0.46 <sup>b</sup>	14.96 ± 1.13 <sup>a</sup>	15 ± 0.6 <sup>a</sup>	15.62 ± 0.85 <sup>a</sup>
Lipid (%)	13.26 ± 1.95 <sup>a</sup>	9.83 ± 1.65 <sup>b</sup>	12.56 ± 1.89 <sup>ab</sup>	13.96 ± 1.3 <sup>a</sup>
Moisture (%)	70.80 ± 3.22	70.66 ± 2.76	70.78 ± 3.7	70.24 ± 3.22
Ash (%)	2.3 ± 0.19 <sup>ab</sup>	2.12 ± 0.04 <sup>ab</sup>	2.4 ± 0.29 <sup>a</sup>	1.98 ± 0.04 <sup>b</sup>

The values with different letters in the same rows are significantly different (Duncan,  $P < 0.05$ )

**Table 2** Fatty acid composition of common carp following a 2-month feeding trial with control diet, turmeric, curcumin, and curcumin nanomicelle (curcumin-NM)

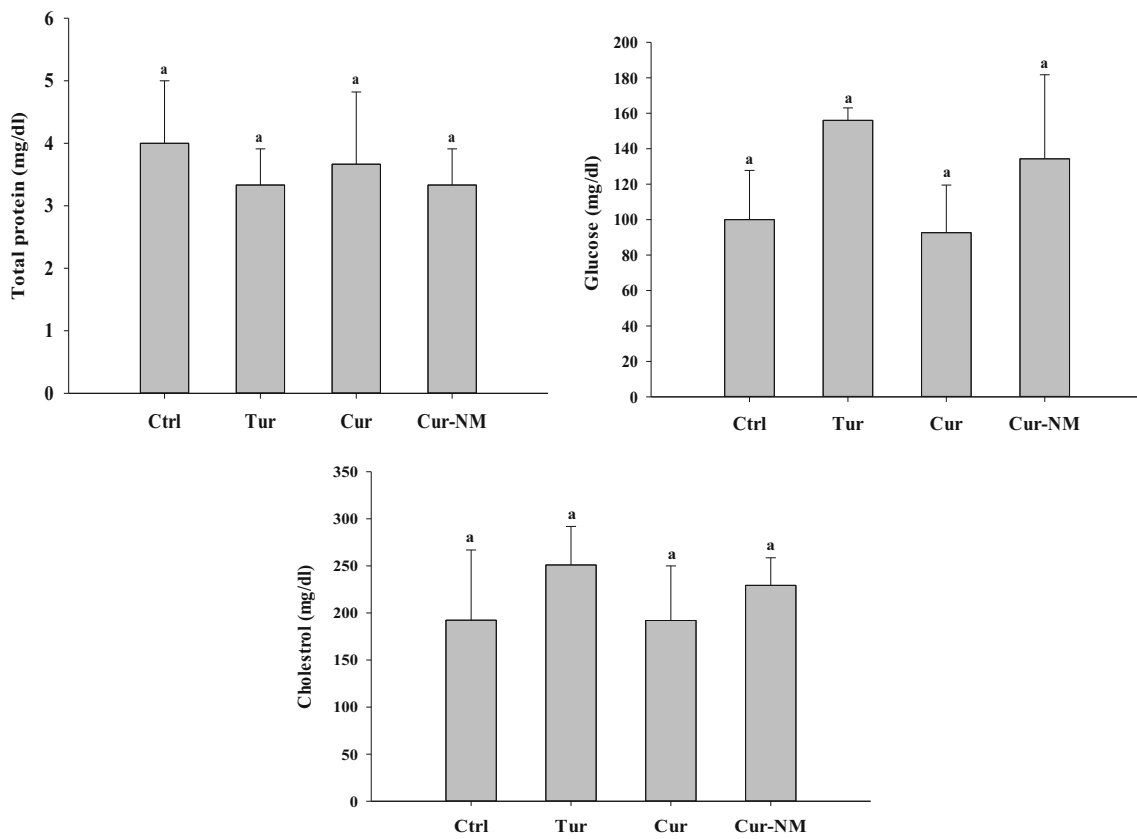
Fatty acid	% of fatty acids/treatment			
	Control	Turmeric	Curcumin	Curcumin-NM
<b>C14:0</b>	0.65 ± 0.10	1.25 ± 0.28	1.19 ± 0.03	1.02 ± 0.69
<b>C15:0</b>	0.29 ± 0.005	0.30 ± 0.007	0.30 ± 0.01	0.38 ± 0.15
<b>C16:0</b>	15.88 ± 0.08 <sup>c</sup>	16.93 ± 0.14 <sup>b</sup>	17.25 ± 0.05 <sup>a</sup>	15.88 ± 0.15 <sup>c</sup>
<b>C17:0</b>	0.22 ± 0.01 <sup>b</sup>	0.54 ± 0.22 <sup>a</sup>	0.23 ± 0.02 <sup>b</sup>	0.46 ± 0.07 <sup>a</sup>
<b>C18:0</b>	4.10 ± 0.03 <sup>b</sup>	4.35 ± 0.05 <sup>a</sup>	4.41 ± 0.03 <sup>a</sup>	3.98 ± 0.02 <sup>c</sup>
<b>C20:0</b>	0.16 ± 0.01	0.19 ± 0.01	0.18 ± 0.03	0.15 ± 0.01
<b>C22:0</b>	0.05 ± 0.003	0.05 ± 0.01	0.04 ± 0	0.05 ± 0.02
<b>C24:0</b>	0.03 ± 0.005	0.03 ± 0.01	0.04 ± 0.0	0.04 ± 0.01
<b>∑SFA</b>	21.42 ± 0.15 <sup>b</sup>	23.57 ± 0.47 <sup>a</sup>	23.65 ± 0.07 <sup>a</sup>	21.99 ± 0.91 <sup>b</sup>
<b>C16:1</b>	4.55 ± 0.10 <sup>b</sup>	4.53 ± 0.24 <sup>b</sup>	4.95 ± 0.04 <sup>a</sup>	4.64 ± 0.12 <sup>b</sup>
<b>C17:1</b>	0.34 ± 0.01 <sup>b</sup>	0.43 ± 0.06 <sup>a</sup>	0.40 ± 0.03 <sup>ab</sup>	0.39 ± 0.04 <sup>ab</sup>
<b>C18:1(n-9)C</b>	39.46 ± 0.23 <sup>c</sup>	40.17 ± 0.40 <sup>b</sup>	42.68 ± 0.09 <sup>a</sup>	38.85 ± 0.20 <sup>d</sup>
<b>C20:1</b>	0.28 ± 0.03 <sup>b</sup>	0.28 ± 0.01 <sup>b</sup>	0.17 ± 0.01 <sup>c</sup>	0.38 ± 0.03 <sup>a</sup>
<b>C22:1</b>	0.32 ± 0.02 <sup>a</sup>	0.25 ± 0.03 <sup>b</sup>	0.20 ± 0.02 <sup>c</sup>	0.31 ± 0.01 <sup>a</sup>
<b>∑MUFA</b>	44.95 ± 0.33 <sup>c</sup>	45.67 ± 0.12 <sup>b</sup>	48.42 ± 0.13 <sup>a</sup>	44.58 ± 0.22 <sup>c</sup>
<b>C18:2 (n-6)C</b>	25.19 ± 0.12 <sup>a</sup>	22.89 ± 0.2 <sup>b</sup>	21.82 ± 0.09 <sup>d</sup>	23.34 ± 0.03 <sup>b</sup>
<b>C18:3 n6</b>	0.22 ± 0.01 <sup>b</sup>	0.21 ± 0.02 <sup>b</sup>	0.14 ± 0.01 <sup>c</sup>	0.26 ± 0.03 <sup>a</sup>
<b>C18:3 n3</b>	1.70 ± 0.02 <sup>b</sup>	1.65 ± 0.01 <sup>b</sup>	1.34 ± 0.05 <sup>a</sup>	1.85 ± 0.04 <sup>a</sup>
<b>C20:2</b>	1.58 ± 0.03 <sup>a</sup>	1.41 ± 0.01 <sup>b</sup>	1.54 ± 0.05 <sup>a</sup>	1.41 ± 0.08 <sup>b</sup>
<b>C20:3 n6</b>	0.30 ± 0.02 <sup>a</sup>	0.23 ± 0.02 <sup>b</sup>	0.24 ± 0.04 <sup>b</sup>	0.32 ± 0.01 <sup>a</sup>
<b>C20:3 n3</b>	0.61 ± 0.03 <sup>a</sup>	0.39 ± 0.01 <sup>b</sup>	0.43 ± 0.03 <sup>b</sup>	0.46 ± 0.10 <sup>b</sup>
<b>C20:4n6 ARA</b>	0.40 ± 0.02 <sup>c</sup>	0.51 ± 0.03 <sup>b</sup>	0.32 ± 0.03 <sup>c</sup>	0.64 ± 0.06 <sup>a</sup>
<b>C20:5n3 EPA</b>	0.83 ± 0.08 <sup>b</sup>	0.81 ± 0.005 <sup>b</sup>	0.58 ± 0.04 <sup>c</sup>	1.10 ± 0.05 <sup>a</sup>
<b>C22:4n6 DTA</b>	0.06 ± 0.002 <sup>c</sup>	0.08 ± 0.02 <sup>b</sup>	0.4 ± 0.01 <sup>b</sup>	0.16 ± 0.02 <sup>a</sup>
<b>C22:5 n6</b>	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01
<b>C22:5n3 DPA</b>	0.30 ± 0.02 <sup>c</sup>	0.23 ± 0.01 <sup>b</sup>	0.20 ± 0 <sup>d</sup>	0.35 ± 0.01 <sup>a</sup>
<b>C22:6n3 DHA</b>	2.25 ± 0.02 <sup>c</sup>	2.12 ± 0.04 <sup>b</sup>	1.19 ± 0.03 <sup>b</sup>	3.30 ± 0.58 <sup>a</sup>
<b>∑PUFA</b>	33.53 ± 0.06 <sup>a</sup>	30.62 ± 0.18 <sup>c</sup>	27.93 ± 0.05 <sup>d</sup>	33 ± 0.10 <sup>b</sup>

The values with different letters in the same rows are significantly different (Duncan,  $P < 0.05$ )

The present study showed that curcumin and curcumin-NM did not improve the WG in comparison with the control group. These findings are in agreement with our previous results in rainbow trout (Kohshahi et al. 2019). The absence of a marked difference between fish fed curcumin and curcumin-NM in comparison with the control group may be due to the taste of curcumin which may be a factor causing lower feed intake and growth rate (Hosseini-Vashan et al. 2012). Recently, a quantitative review evaluated the results of 13 studies derived from turmeric and curcumin and showed that higher levels of curcumin in the diet as the main bioactive substance of turmeric can potentially cause inflammation and lower body weight (Fagnon et al. 2020; Yusuf et al. 2017)

In the present study, HSI decreased significantly in fish fed dietary turmeric and curcumin-NM supplementation. HSI is a good indicator of feeding activity and provides information

about the liver, and the lower HSI could be attributed to decreased liver glycogen and fat deposition as described by Liu et al. (2020). In contrast to our results, however, no significant change was observed in HSI of African catfish, crucian carp, *Astyanax aff. bimaculatus*, and genetically improved farmed tilapia (GIFT) (Cui et al. 2013; Ferreira et al. 2017; Jiang et al. 2016; Rawung et al. 2020). Normally, the body composition is affected by the diet and feeding rate, genetics, and rearing conditions (Dumas et al. 2007). Proximate analysis could be a good indicator of fish growth (Ali et al. 2005). Supplementation of turmeric, curcumin, and curcumin-NM caused a significant increase in the crude protein. Similar to our results, Hwang et al. (2013) found that dietary turmeric produced a higher protein content in the body composition of fish that were fed with a higher level of turmeric. Moreover, Mahmoud et al. (2014) reported that dietary turmeric resulted

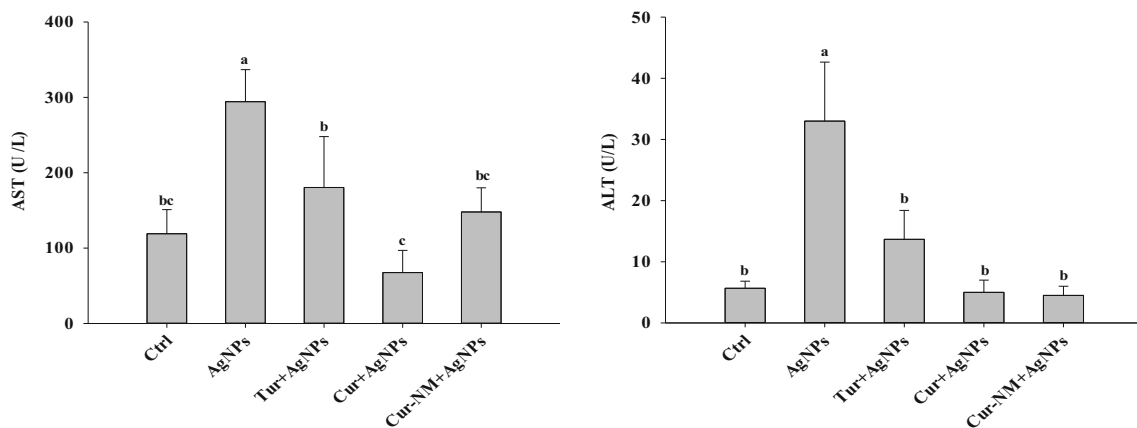


**Fig. 3** The levels of glucose, cholesterol, and total protein in the plasma of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet (Ctrl), turmeric (Tur), curcumin (Cur), and curcumin

nanomicelle (Cur-NM). Bars with different letters are significantly different (mean ± SD, Duncan,  $P < 0.05$ )

in a significantly higher protein content, but produced a lower total lipid with the increase of the turmeric level in the diet. In the present study, the levels of protein and lipid could be affected by the level of turmeric which consequently stimulates synthesis of muscle protein and lipid contents. However,

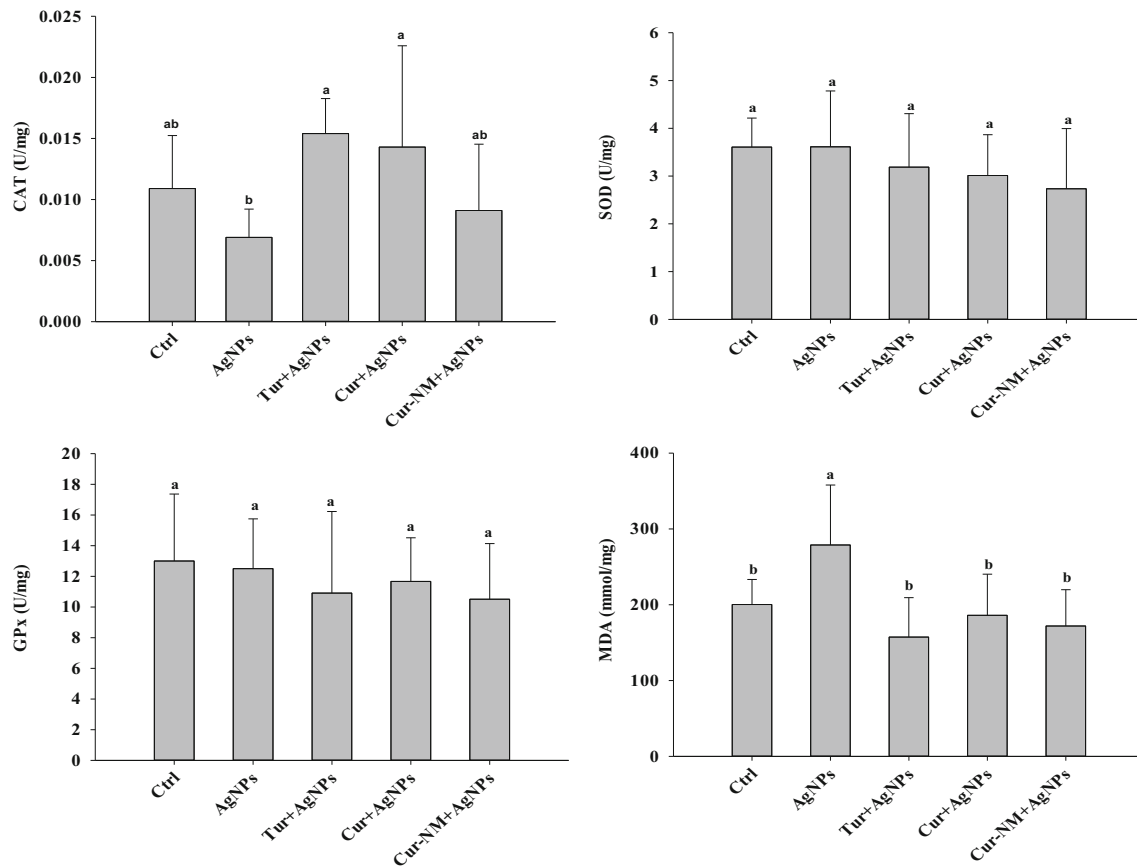
some other studies have shown that the dietary turmeric had no effects on the proximate composition of common carp and rainbow trout (Abdel-Tawwab and Abbass 2017; Kohshahi et al. 2019). The increasing level of crude protein could be related to the stimulation effect of curcumin and curcumin-



**Fig. 4** The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the plasma of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet, turmeric, curcumin, and curcumin nanomicelle and 96-h exposure to silver nanoparticles (AgNPs, 0.5 mg L<sup>-1</sup>). Ctrl, fed on control diet and was not exposed to AgNPs; AgNPs, fed on control diet and exposed to AgNPs; Tur +

AgNPs, fed on diet containing turmeric and exposed to AgNPs; Cur + AgNPs, fed on diet containing curcumin and exposed to AgNPs; Cur-NM + AgNPs, fed on diet containing curcumin nanomicelle and exposed to AgNPs. Bars with different letters are significantly different (mean ± SD, one-way ANOVA,  $P < 0.05$ )





**Fig. 5** The levels of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and malondialdehyde (MDA) in the liver of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet, turmeric, curcumin, and curcumin nanomicelle and 96-h exposure to silver nanoparticles (AgNPs,  $0.5 \text{ mg L}^{-1}$ ). Ctrl, fed on control diet and was not exposed to AgNPs; AgNPs, fed on control diet and

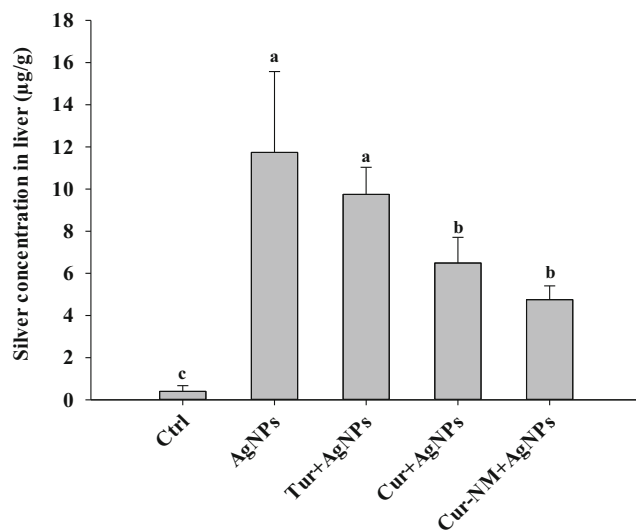
exposed to AgNPs; Tur + AgNPs, fed on diet containing turmeric and exposed to AgNPs; Cur + AgNPs, fed on diet containing curcumin and exposed to AgNPs; Cur-NM + AgNPs, fed on diet containing curcumin nanomicelle and exposed to AgNPs. Bars with different letters are significantly different (mean  $\pm$  SD, one-way ANOVA,  $P < 0.05$ )

NM on digestive enzymes such as trypsin and lipase (Jiang et al. 2016). Similarly, previous studies showed that dietary curcumin ( $50 \text{ mg kg}^{-1}$ ) and turmeric (0.5%) could effectively increase the protein content of the body (Mahmoud et al. 2017; Mahmoud et al. 2014). The level of ash was found significantly higher in the curcumin group ( $2.4 \pm 0.29\%$ ) than fish fed curcumin-NM ( $1.98 \pm 0.04\%$ ). This may be related to the ability of curcumin to bind with metal ions to offer antioxidant activity (Began et al. 1999).

The mean concentrations of SFA and MUFA have increased following 2 months feeding trial with turmeric and curcumin. Furthermore, dietary curcumin showed an ability to reduce the muscular PUFA content. Generally, the fatty acid composition depends on the species and their diets (Ackman 2007). A higher concentration of PUFA in the fish meat can cause oxidation as well as oxidative stress which consequently a higher amount of antioxidant must be added into diets to prevent the effects of oxidative stress. Several studies have reported that curcumin might be involved in the

biosynthesis of fatty acids, and its antioxidant property indirectly can affect the meat fatty acid profile (Rukkumani et al. 2003; Salah et al. 2019). However, there are few studies focused on consumption of curcumin in fish and its mechanism of action on the fatty acid profile.

It has been shown that dietary turmeric reduces lipid peroxidation by increasing the activities of antioxidant enzymes (Reddy and Lokesh 1994). Also, curcumin can potentially increase the content of PUFA by blocking lipid peroxidation. Our results showed that the total SFA content of fish fed turmeric and curcumin increased significantly compared to the control group. This might be related to the action of curcumin in stimulating the activity of enzymes involved in the production of SFA from unsaturated fatty acids (Kaul and Krishnakantha 1997). In other vertebrates, the effects of dietary turmeric on fatty acid composition have also been investigated. Daneshyar et al. (2011) reported that supplementation of 0.75% turmeric in the diet of broiler chicken significantly decreased the total SFA and trans-vaccenic acid (C18:1n7) of



**Fig. 6** Silver accumulation in the liver tissue of common carp (*Cyprinus carpio*) following a 2-month feeding trial with control diet, turmeric, curcumin, and curcumin nanomicelle and 96-h exposure to silver nanoparticles (AgNPs,  $0.5 \text{ mg L}^{-1}$ ). Ctrl, fed on control diet and was not exposed to AgNPs; AgNPs, fed on control diet and exposed to AgNPs; Tur + AgNPs, fed on diet containing turmeric and exposed to AgNPs; Cur + AgNPs, fed on diet containing curcumin and exposed to AgNPs; Cur-NM + AgNPs, fed on diet containing curcumin nanomicelle and exposed to AgNPs. Bars with different letters are significantly different (mean  $\pm$  SD, one-way ANOVA,  $P < 0.05$ )

thigh muscles, but total unsaturated fatty acids in contrast to our results, remained unchanged.

Also, Peiretti et al. (2011) showed that adding  $3 \text{ g kg}^{-1}$  turmeric diet led to higher levels of  $\alpha$ -linolenic acid and PUFA n-3 in the meat of the rabbits, and therefore improved the meat quality.

The biochemical parameters (i.e., AST and ALT) after the exposure experiment showed a remarkable reduction in fish with pre-feeding trial experience in comparison to the positive control group. The serum levels of AST and ALT provide vital information about the liver disorders. It has been shown that higher concentrations of AST and ALT may reflect stress-based tissue injuries (Huang et al. 2006). Previous studies have demonstrated that AgNPs have direct effects on the elevated level of AST and ALT (Kumar et al. 2018; Ramachandran et al. 2018). This might be due to the release of these enzymes to the bloodstream, after the interaction of hepatocytes by AgNPs derived free radicals (Martínez-Gutiérrez et al. 2012). However, the decreased levels of AST and ALT during the exposure experiment may be a sign of antioxidant activity of turmeric powder and its derivatives (Saccol et al. 2017). Our results are similar to that reported by El-Houseiny et al. (2019) who found African catfish (*Clarias gariepinus*) fed on 5% turmeric showed significantly lower values of ALT and ALP activities.

Awasthi et al. (2019) showed the protective role of curcumin against  $\text{Cr}^{6+}$ -induced hepatotoxicity, genotoxicity,

and oxidative stress in spotted snakehead (*Channa punctatus*) fish. Based on our results, no significant differences were observed in activity of GPx and SOD after 96-h exposure to AgNPs. Previous studies have also reported similar finding (Ale et al. 2018; Choi et al. 2010; Ferreira et al. 2017; Kumar et al. 2018). On the other hand, the activity of CAT following 96-h exposure to AgNPs was significantly lower in fish fed on basal diet and exposed to AgNPs. The reduced level of CAT activity may be influenced by the environmental stressors, and liver's failure to provide an efficient antioxidant defense system (Wu and Zhou 2013). Similar to our results, Govindasamy and Rahuman (2012) reported that CAT activity was significantly decreased in liver tissues of Mozambique tilapia (*Oreochromis mossambicus*) fed on  $0.5 \text{ mg kg}^{-1}$  Se, and the lowest MDA level was observed in the  $0.48 \text{ mg kg}^{-1}$  Se group. Similarly, Choi et al. (2010) has also reported dose-dependent decrease in CAT activity in zebrafish liver following exposure to AgNPs. The results obtained from the CAT activity is in agreement with those obtained by El-Houseiny et al. (2019), who reported that the value of CAT activity in the serum African catfish increased remarkably in fish fed diets supplemented with 0.5% turmeric. However, Ferreira et al. (2017) showed that dietary turmeric did not affect the CAT activity in *Astyanax aff. bimaculatus*.

In this study, the highest level of MDA content was observed in fish fed on basal diet and exposed to AgNPs. MDA is one the final products of lipid peroxidation and its measurement as an index of lipid peroxidation provides vital information about the meat quality (Gatta et al. 2000; Tsikas 2017). In the present study, the level of MDA in the liver was significantly decreased in fish fed on turmeric, curcumin and curcumin-NM after exposure to AgNPs. The decrease in the MDA levels seems to be due to the ability of curcumin to scavenge free radicals and enhance the antioxidant system against oxidative stress (Somparn et al. 2007). Our results are in agreement with those obtained by El-Houseiny et al. (2019) who reported that 0.5% turmeric in diet of cadmium-induced fish caused a significant decrease in serum MDA level. Meanwhile, Ferreira et al. (2017) reported no significant changes in liver MDA level of *Astyanax aff. bimaculatus* fed with dietary turmeric. Recently, Rajabiesterabadi et al. (2020) showed that administration of  $10 \text{ g kg}^{-1}$  turmeric diet would be effective to protect the common carps against oxidative damage cause by copper exposure.

Silver accumulation in the liver after 96-h of exposure showed significant changes between the AgNP-exposed fish. Curcumin-NM and curcumin groups exhibited significant decreases in liver Ag accumulation. However, slightly higher accumulation occurred in the curcumin group. Environmental pollutants are generally accumulated in the fish organs, causing several health hazards such as hepatotoxicity (Costa et al. 2010) and oxidative damages (Gagné et al. 2012). It has been shown that AgNPs are mainly accumulated

in the liver (Wu and Zhou 2013). This is most likely to be dependent on chemical structure of AgNPs (Navarro et al. 2008), different uptake pathways (Wu and Zhou 2013) and species-specific factors. Although it has been shown that supplementation of turmeric powder in the diet reduced the amount of cadmium in the liver (El-Houseiny et al. 2019). However, in the present study, turmeric could not prevent Ag accumulation in the liver of exposed fish in compare to the curcumin-NM and curcumin group. It has been shown that the protective activity of turmeric in ingesting the toxic metals could be associated with the presence of curcuminoids and their different binding sites such as carboxyl, phenolic, and amines/hydroxyl groups (Qayoom et al. 2017). Furthermore, previous studies have been suggested that these groups are all involved in metal-ligand interface which results in lower bioaccumulation of metals (Anayurt et al. 2009; Veglio et al. 1997).

## Conclusion

In summary, the present study shows that supplementation of turmeric ( $50 \text{ g kg}^{-1}$ ) or curcumin ( $1000 \text{ mg kg}^{-1}$ ) in diet resulted in a positive effect on weight gain and fatty acid composition in the common carp. However, these findings rejected our initial hypothesis that the nanoencapsulated form of curcumin is more effective than free curcumin as the growth and biochemical parameters did not improve in the curcumin-NM group. Although the exposure experiment showed that AgNP induces oxidative stress and hepatotoxicity in carps, the biochemical parameters after exposure experiment demonstrated that dietary turmeric and curcumin might be effective to protect the fish against AgNPs. This study provides interesting evidence that supplementation of curcumin-NM to the diet can potentially protect the liver against the bioaccumulation of AgNPs. However, further work is needed to understand the mechanism of dietary curcumin-NM on growth performances in different fish species. Thus, the results of the present study suggest adding  $50 \text{ g kg}^{-1}$  turmeric or  $1000 \text{ mg kg}^{-1}$  curcumin diet to enhance growth performances, health, and meat quality of juvenile carps.

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**Availability of data and materials** All data and materials are included in this published article.

**Author contribution** FP: investigation, data curation; SM: investigation; SA: investigation, data curation, writing—original draft preparation; SAJ: supervision, conceptualization, methodology, funding acquisition, project administration, writing—review and editing; EG: investigation; HPK: investigation; IJY: conceptualization, methodology

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## Declarations

**Ethics approval** All animal manipulation procedures were done based on Animal Welfare Act and Interagency Research Animal Committee guidelines (Nickum et al. 2004).

**Consent to participate** Not applicable

**Consent for publication** Not applicable

**Competing interests** The authors declare no competing interests.

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