

Original Article

Effect of different levels of chitosan dietary supplement on heavy metal accumulation and growth performance in common carp, *Cyprinus carpio*

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Abstract: This study was conducted to examine the effects of different levels of chitosan dietary supplementation on heavy metal accumulation and growth performance of the common carp in the Al-Gharraf River, the main tributary of the Tigris River, which extends from the right bank of the Tigris at Al-Kut Dam to the Hamar Marsh. After chitosan supplementation, 200 fish (90 ± 10 g) were reared in floating cages in the Al-Gharraf River, Al-Rifai region. The fish were divided into four treatments: a control group without chitosan, a 1% chitosan group, a 2% chitosan group, and a 3% chitosan group. The rearing period began in April 2024 and lasted 4 months. The results indicated that chitosan significantly enhanced the deposition of Pb and Cd in the serum and muscle of fish, resulting in lower levels as the chitosan concentration increased. In addition, increasing the chitosan concentration improved fish growth parameters.

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Introduction

The Al-Gharraf River is susceptible to heavy metal pollution due to human activities. Many activities, such as industrial and food processing plants, release heavy metals into aquatic ecosystems, a situation exacerbated by domestic and agricultural wastes (Al-Rudaini and Al-Samawi, 2017; Zhang et al., 2023; Al-Salman, 2023; Wei et al., 2024; Al-Saadi et al., 2024; Alkarbolii and Hamood, 2025). Heavy metals are characterized by their toxicity, environmental persistence, and potential for bioaccumulation, posing significant risks to ecosystems (Hashem and Abdul-Jalil, 2024). Therefore, assessing metal pollution and identifying its sources is of great importance (Shin et al., 2019; Fadel and Abdul-Hussein, 2022; AlSalman and Dawood, 2023).

The common carp (*Cyprinus carpio*), a widely consumed freshwater fish species, is notably susceptible to heavy metal accumulation, raising serious concerns about its safety for human consumption. It is easily identified by its laterally compressed body, three rows of pharyngeal teeth, two pairs of barbels, and an elongated dorsal fin

characterized by three or four simple rays. Both the dorsal and anal fins exhibit distinct markings (Balloon, 1975; Hussein et al., 2016). Even minor exposure to heavy metals can adversely affect various fish organs, particularly those associated with the nervous, reproductive, and respiratory systems (Mostafa et al., 2020; AlSalman and Dawood, 2021; Aziz et al., 2023; Saad and Hamood, 2025). Monitoring heavy metal levels in commonly consumed fish species is essential due to the associated health risks, necessitating the implementation of pollution mitigation measures (Al-Hamed, 2020).

Developing strategies to reduce heavy metal concentrations in water and their bioaccumulation in fish is critical (Ibrahim and Ibrahim, 2013). Chemical precipitation, ion exchange, and activated carbon adsorption are traditional techniques for removing heavy metals from water (AlSalman and Dawood, 2023). However, these methods can be expensive and may add additional pollutants. Bioremediation, on the other hand, effectively removes heavy metals from contaminated water sources using biological agents,

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Table 1. The composition of the diets used in the experiment in 100 kg (NRC, 2011).

Ingredients	Control 0% Chitosan	T1 1% Chitosan	T2 2% Chitosan	T3 3% Chitosan
Wheat flour	15.0	14.5	14.0	13.5
Yellow Corn	16.0	15.5	15.0	14.5
Bran	15.0	15.0	15.0	15.0
Soybean meal	25.0	25.0	25.0	25.0
Fish meal	25.0	25.0	25.0	25.0
Garlic + enzymes + minerals	1.0	1.0	1.0	1.0
Vegetable oil	3.0	3.0	3.0	3.0
Chitosan	0.0	1.0	2.0	3.0
Total /kg	100.0	100.0	100.0	100.0
Crud protein %	31.49	31.39	31.28	31.18
Gross energy (kcal/ kg)	2825.3	2793.0	2760.8	2728.55

such as chitosan (Taha et al., 2019). Therefore, this study was conducted to examine the effects of different levels of chitosan dietary supplementation on heavy metal accumulation and growth performance of the common carp in the Al-Gharraf River, the main tributary of the Tigris River, which extends from the right bank of the Tigris at Al-Kut Dam to the Hamar Marsh.

Materials and Methods

A total of 200 fish were obtained from a farm with floating cages in the Gharraf River located in the Al-Rafai district. The fish, with an average weight of about 90 ± 10 g, were transported to 4 floating cages installed in the Gharraf River and acclimatized to the experimental conditions for 2 weeks. During this period, fish were fed a diet similar to the control group (Table 1) (3% biomass), and water temperature and pH were 25.3°C and 7.8, respectively. Four treatments, including (1) control without chitosan supplementation, (2) 1% chitosan, (3) 2% chitosan, and (4) 3% chitosan, were designed. Fish were hand-fed twice daily at a rate of 3% of body weight. The experiment lasted 4 months, starting in August 2024. Proximate composition of the feed ingredients and treatments were given in Table 1. The weight of the fish was measured using an electronic balance LC400 (LIBRONIC, Germany).

Heavy metal measurement: The trace elements of lead and cadmium were extracted from the water after digestion using the APHA (2005) method. They were calculated using the calibration curve according to

Usco (1992). Trace elements in water were calculated using the equation: $E_{con} = A \times B / C \times 1000$, where E_{con} = concentration of the element in water ($\mu\text{g/L}$), A = concentration of the element extracted from the calibration curve ($\mu\text{g/l}$), B = final volume of the filter sample (ml) and C = Initial volume of the filter sample (ml). These metals were measured in the fish muscle using the equation: $E_{con} = AB / D$, where E_{con} = concentration of the element in the sample ($\mu\text{g/g}$ dry weight), A = concentration of the element extracted from the calibration curve ($\mu\text{g/l}$), B = final volume of the filter sample (ml), and D = dry weight of the sample (g).

Chitosan treatment: To ensure consistent consumption, fish in the chitosan-supplemented groups were given daily doses of organic chitosan mixed into their feed.

Blood samples: Blood samples were collected using a 3 ml plastic syringe from the caudal vein of fish cultivated in floating cages in the Al-Gharraf River. Each blood sample was collected in a single gelatin tube without an anticoagulant. The samples were centrifuged at 6,000 rpm for 5 minutes at 4°C . Subsequently, the serum was isolated and stored at -20°C for further quantification of Pb and Cd in the blood serum.

Muscle samples: The fish were dispatched for their muscles, which were cut into small portions and left in the sun to be dried for a week. Subsequently, they were sent to the laboratories for the measurement of Pb and Cd levels using the method mentioned above.

Table 2. The effect of chitosan on the Pb concentrations in the fish's muscles and blood serum from four studied treatments.

Groups	Pb/muscles mg/kg	Pb Serum Ppb
Control: 0% Chitosan	2.00±0.23a	12.98±0.80a
T1: 1% Chitosan	1.32±0.14b	7.63±0.49b
T2: 2% Chitosan	0.08±0.03c	3.70±0.19c
T3: 3% Chitosan	0.02±0.003c	3.08±0.17c
LSD	0.38	1.37

Means with a different small letter in the same column are significantly different ($P<0.05$).

Table 3. The effect of chitosan on the reduction percentage of Pb in muscles and serum across different groups. Values are reported as mean±SE.

Groups	Pb/muscles%	Pb Serum %
T1: 1% Chitosan	34.00±1.15b	42.10±1.55b
T2: 2% Chitosan	96.00±0.57a	71.60±2.22a
T3: 3% Chitosan	99.00±1.73a	76.35±1.36a
LSD	4.31	7.45

Means with a different small letter in the same column are significantly different ($P<0.05$).

Table 4. The effect of chitosan on the Cd concentrations in the fish's muscles and serum from different groups. Values are reported as mean±SE.

Groups	Cd muscles(mg/kg)	Cd Serum (Ppb)
Control (0% Chitosan)	0.10±0.01a	1.90±0.21a
T1 (1% Chitosan)	0.07±0.01a	1.61±0.18a
T2 (2% Chitosan)	0.03±0.01b	0.96±0.08b
T3 (3% Chitosan)	0.01±0.002b	0.50±0.05c
LSD	0.03	0.42

Means with a different small letter in the same column are significantly different ($P<0.05$).

Growth parameters: The growth parameters were calculated using the following equations (Jobling, 1993):

Total weight gain (W g) = FW – IW

Daily gain (Total gain, g/days) = (FW – IW) / days

Relative growth rate (RGR, %) = $100 \times (FW - IW) / IW$

Specific growth rate (SGR, %/day) = $100 \times (\ln FW - \ln IW) / \text{days}$

Reduction% = (treat-control)/control * 100

Statistical analysis: The results are presented as the mean±standard error. The statistical program SAS (Statistical Analysis System, version 9.1) was used to analyze the data. The significance of the differences between the means was determined using the Least Significant Differences (LSD) post hoc test and the one-way ANOVA at the $P \leq 0.05$ significance level.

Results

Pb concentrations: Fish in the 3% chitosan treatment had significantly lower Pb levels in their muscles and serum ($P<0.05$) than the control and other groups (Table 2). Treatment with 3% chitosan

supplementation resulted in a significant reduction in Pb accumulation in muscle and serum ($P<0.05$) compared with the control and other groups (Table 3).

Cd concentrations: The levels of Cd in the muscles and serum of fish in the treatment group that received a 3% chitosan additive were significantly reduced ($P<0.05$) compared with the control and other groups (Tables 4, 5). The group containing 3% chitosan complies with the Pb concentrations Guidelines of the Codex Alimentarius Commission (FAO/WHO, 2019). All results indicate that the permissible limit established by these guidelines is not exceeded (Table 6). As reported by the Food and Agriculture Organisation of the United Nations (2024), Cd concentrations in the groups containing 2 and 3% chitosan, respectively, are within permissible limits (Table 7).

Growth parameters: The results revealed a significant increase in weight gain and body weight ($P<0.05$) in the 3% chitosan treatment compared to the control and other groups, with values of 1660.54 grams and 1758.74 grams, respectively (Table 8). The findings indicate a significant enhancement ($P<0.05$)

Table 5. The effect of chitosan on the reduction in Cd concentration in muscles and serum across different groups. Values are reported as mean±SE.

Groups	Cd/muscles%	Cd Serum %
T1: 1% Chitosan	30.43±2.31c	43.05±3.18c
T2: 2% Chitosan	70.27±1.54b	49.49±0.28b
T3: 3% Chitosan	99.00±1.57a	86.75±0.27a
LSD	9.71	6.40

Means with a different small letter in the same column are significantly different ($P<0.05$).

Table 6. Lead concentrations in the fish muscles in different groups compared with the Codex Alimentarius Commission (FAO/WHO). (2019).

Lead mg/kg In muscles			
Above 0.3	44	39	2
lower than 0.3	6	11	48
Yates' Chi-square value	128.88		
Yates' P-value	<0.0001		

Table 7. Cadmium concentrations in the fish muscles in different groups compared with those of the Food and Agriculture Organization of the United Nations. (2024).

Cadmium mg/kg in muscles			
Above 0.05	24	17	1
lower than 0.05	26	33	49
Yates' Chi-square value	43.46		
Yates' P-value	<0.0001		

Table 8. Some growth parameters, including initial weight, Weight gain, and body weight (g), in different groups at the end of the experiment. Values are reported as mean ± SE.

Groups	Initial weight (g)	Final body weight (g)	Weight gain (g)
Control: 0% Chitosan	98.44±0.36	1487.72±25.96c	1389.28±25.63c
T1: 1% Chitosan	98.46±0.38	1590.44±24.33b	1491.98±24.36b
T2: 2% Chitosan	98.68±0.35	1726.26±22.42a	1627.58±22.50a
T3: 3% Chitosan	98.20±0.38	1758.74±41.51a	1660.54±25.28a
LSD	1.04	68.28	68.26

Means with a different small letter in the same column are significantly different ($P<0.05$).

in daily weight gain, relative growth rate, and specific growth rate in the 2% group when compared to the control and other groups, with recorded values of 13.83, 1691.62, and 2.40 g, respectively (Table 9).

Discussions

Heavy metal concentrations in fish muscles and blood serum: The results revealed that supplementation of chitosan into fish diets significantly reduced Pb levels in both muscle and blood serum. Increasing the chitosan concentration in the diet to 3% resulted in a significant decrease in Pb level in both the muscles and the serum of the treated group. The protective function of chitosan in mitigating lead toxicity is linked to its mechanism (Gopinath and Ravichandran, 2020), which interacts

with lead in the gastrointestinal tract, reducing its absorption into the bloodstream and tissues. Tunsoy and Duran (2020) reported reduced accumulation of heavy metals in *Clarias gariepinus* exposed to Pb and supplementation of a chitosan mixture, demonstrating a significant reduction in lead concentrations in its gill tissue.

Cadmium concentrations decreased with increasing chitosan supplementation compared to the control group. Chitosan, like Pb, binds to Cd in the gastrointestinal tract, reducing its absorption into the bloodstream and tissues. Previous studies demonstrate that dietary chitosan reduces Cd accumulation (Kim et al., 2016; Salam et al., 2021). Low-molecular-weight chitosan enhances antioxidant markers, suggesting a potential protective role against Cd-induced oxidative

Table 9. Growth parameters of the common carp. Values are reported as mean±SE.

Groups	Daily weight gain /	Relative growth	Specific growth
Control: 0% Chitosan	11.57±0.21c	1411.56±25.98c	2.25±0.01c
T1: 1% Chitosan	12.43±0.20b	1516.87±25.98b	2.31±0.01b
T2: 2% Chitosan	13.83±0.21a	1691.62±25.85a	2.40±0.01a
T3: 3% Chitosan	13.56±0.18a	1651.39±25.01a	2.38±0.01a
LSD	0.56	71.71	0.03

Means with a different small letter in the same column are significantly different ($P<0.05$).

stress, and improves the morphology of the liver and kidneys. Chitosan supplementation in the diet markedly reduced liver fat content and improved kidney morphology compared with a basal diet (Salam et al., 2021). Al-Najjar et al. (2021) indicate that different fish species may exhibit varying responses to chitosan supplementation regarding Cd accumulation.

Elevating the chitosan content in the diet significantly increases the proportion of fish with Pb concentrations below 0.3 mg/kg and Cd levels below 0.05 mg/kg. The Iraqi standard for Pb in fish is set at 0.3 mg/kg, while the standard for cadmium is 0.05 mg/kg, both of which represent critical thresholds. Chitosan treatments at 2 and 3% significantly increase the number of fish meeting this standard. A dose-response relationship is evident, demonstrating that higher chitosan concentrations significantly decrease muscle Pb and Cd levels. Al-Aboudi et al. (2022) reported the accumulation of Pb and Cd in the tissues of common carp in the cages in the Garraf River, revealing that metal accumulation in fish tissue occurs in the following order: kidney > gills > muscles. This indicates that while muscles accumulate Pb and Cd, other organs exhibit higher concentrations of these metals. This work corroborates earlier findings regarding the Al-Gharraf River (Al-Janabi et al., 2021; Al-Aboudi et al., 2022), and presents compelling evidence of heavy metal contamination in fish muscle. Chitosan effectively reduces lead and cadmium levels in fish muscle tissue, aiding compliance with WHO standards.

Growth parameters: All groups had similar initial weights (98.20-98.68 g), with no significant differences. Based on the results, 3% chitosan treatment exhibited the greatest weight gain and final body weight, with significant differences compared to

the control and other experimental groups. The control group showed the lowest weight gain and final body weight, significantly lower than those of the treated fish with chitosan. The treatments receiving 2% and 3% chitosan showed the greatest weight gains, at 1627.58 g and 1660.54 g, respectively, with no significant difference between them. Increasing chitosan concentration in the feed improves the absorption of essential nutrients, including fats and proteins (Xie, 2014). In addition, elevated dietary chitosan levels predominantly enhance hypolipidemic effects in connective tissue (Salam et al., 2021). The results of the current work align with those of Chen et al. (2019) and Ismael et al. (2021). Salam et al. (2021) found that a chitosan dosage of 1 g/kg yielded the greatest benefits in silver barb (*Barbonymus gonionotus*), demonstrating its safe use in aquaculture. Chitosan supplementation at 2% effectively enhances growth in common carp, resulting in a 17.2% increase in weight gain, attributed to improved nutrient absorption, immune response, and pathogen management. The absence of a significant difference between the 2% and 3% groups corroborates the principle of diminishing returns at higher doses (Salam et al., 2021). A 2% chitosan concentration is recommended as the economically viable standard for sustainable aquaculture.

The control group demonstrated the lowest daily weight gain, recorded at 11.57±0.21 g. The 2% chitosan treatment yielded the highest daily weight gain (13.83±0.21 g), which was significantly higher than that of the control and 1% groups. The 3% chitosan (13.56±0.18 g) did not differ significantly from the 2% concentration; however, it was significantly greater than both the control and the 1% concentration. The observed increase in daily weight

gain indicates that chitosan supplementation enhances nutrient utilisation and feed efficiency. The maximum growth rate of 2% suggests an optimal level of inclusion.

RGR demonstrated a similar pattern, increasing with the addition of chitosan. These findings align with previous studies demonstrating that dietary chitosan enhances growth performance by improving immune function, gut health, and nutrient absorption (Hameed and Mahmood, 2025). A decrease of 2 to 3% signifies diminishing returns at higher inclusion levels. SGR improved significantly across all chitosan-supplemented groups, with the highest value observed in the 2% group. This supports the theory that 2% chitosan is optimal for enhancing growth efficiency (Chen and Chen, 2019).

Chitosan supplementation in common carp diets significantly improved their growth performance, with a 2% inclusion level yielding optimal results for DWG, RGR, and SGR. 3% chitosan exhibited significantly improved growth compared to the control one; however, the marginal reduction in efficacy relative to 2% suggests that higher concentrations may not yield additional benefits and could lead to a plateau or reduced effectiveness (Dawood and Al-Saigh, 2015; Salam et al., 2021). A study demonstrated that low to intermediate doses of oral chitosan (1 or 5 g/kg) significantly improved body weight gain and specific growth rate in *Misgurnus anguillicaudatus* (Chen and Chen, 2019). Xie (2014) demonstrated that chitosan inhibits growth at high concentrations.

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