Turkish Journal of Science & Technology
19(2), 379-386, 2024

Research Paper
https://doi.org/10.55525/tjst.1447886

Effect of Dietary Zinc on the Antioxidant Parameters of Juvenile Common Carp (*Cyprinus carpio*)

Shokri Omar Mustafa MUSTAFA[1], Muzaffer Mustafa HARLIOĞLU[2*], Önder AKSU[3], Zahra BATOOL[4]

[1, 2, 4] Department of Fish Cultivation, Fisheries Faculty, Firat University, 23200 Elazığ, TÜRKİYE
[3] Department of Fish Cultivation Fisheries Faculty, Munzur University, 62000 Tunceli, TÜRKİYE
[1] shokri.mustafa@uqconnect.edu.au, [2*] mharlioglu@firat.edu.tr, [3] onderaksu@munzur.edu.tr,
[4] mushhood4@gmail.com

(Geliş/Received: 06/03/2024; Kabul/Accepted: 26/07/2024)

Abstract: Zinc (Zn) is an essential micro mineral needed for the proper growth and immune function of fish. This investigation was designed to examine the antioxidant role of a fortified diet with different Zn levels in the muscle and liver tissues of carp fry. A four-iso-nitrogen (35% crude protein) practical diet was produced that included graded levels of dietary zinc sulphate as a nutritional zinc resource in the fundamental diet supplemented with increased zinc levels (T1, control, 85 mg Zn kg^{-1}, T2 105 mg Zn kg^{-1}, T3, 125 mg Zn kg^{-1} and T4, 145 mg Zn kg^{-1}). Even though the SOD and CAT analysis results did not show a linear increase in the increasing Zn ratio in the diets, higher values were obtained compared to the control groups. SOD highest values in T3 for the liver (0.713 ± 0.220 U/ml) and T1 for muscle (0.751 ± 0.144 U/ml), CAT values were highest in T2 for the liver (0.849 ± 0.115 nmol/dk/m) and T2 for muscle (1.059 ± 0.148 nmol/dk/m) was obtained. MDA values were completely higher for the muscle than for the control group, and for the liver, a lower value was obtained in the T2 trial group than in the control group (1.671 ± 0.230 µM). The results of the study showed that Zn contributed significantly to the nutrition of carp fish. It can be concluded that the findings of SOD and CAT analysis endorse the positive contributions of using 105 mg Zn in the diets to promote the antioxidant defense of juvenile carp fish.

Keywords: Antioxidant, catalase, liver, muscle, superoxide dismutase.

Diyetsel Çinkonun Yavru Sazanların (*Cyprinus carpio*) Antioksidan Parametreleri Üzerine Etkisi

Öz: Çinko (Zn), balığın dengeli büyümesi ve metabolizması için gerekli olan önemli bir mikromineraldir. Bu araştırma, sazan yavrularının kas ve karaciğer dokularında farklı Zn düzeylerine sahip zenginleştirilmiş bir diyetin antioksidan rolünü incelemek üzere tasarlanmıştır. Artan çinko seviyeleri ile desteklenen temel diyette besinsel bir çinko kaynağı olarak kademeli seviyelerde diyet çinko sülfat içeren dört izo-nitrojenli (%35 ham protein) pratik bir diyet üretildi (T1, kontrol, 85 mg Zn kg^{-1}, T2 105 mg Zn kg^{-1}, T3, 125 mg Zn kg^{-1} ve T4, 145 mg Zn kg^{-1}). Çalışma sonunda rasyonlarda artan Zn oranında SOD ve CAT analiz sonuçları doğrusal bir artış göstermese de kontrol gruplarına göre daha yüksek değerler elde edilmiştir. En yüksek SOD değerleri karaciğer için T3'te (0,713±0,220 U/ml) ve T1'de kas için (0,751±0,144 U/ml), CAT değerleri en yüksek karaciğer için T2'de (0,849±0,115 nmol/dk/m) ve T2'de kas için (1,059±0,148 nmol/dk/m) elde edildi. MDA değerleri kas için kontrol grubuna göre tamamen yüksekti ve karaciğer için T2 deneme grubunda kontrol grubuna göre daha düşük bir değer elde edildi (1,671 ± 0,230 µM). SOD ve CAT analiz bulgularının, yavru sazan balıklarının antioksidan savunmasını geliştirmek için rasyonlarda 105 mg Zn kullanımının olumlu katkılarını desteklediği sonucuna varılabilir.

Anahtar Kelimeler: Antioksidan, katalaz, karaciğer, kas, süperoksit dismutaz.

1. Introduction

In the production of cultured fish, feed is very important in decisive the quality and quantity of the product obtained. Excess minerals in feeds in dense culture systems are an important problem causing eutrophication. In order to prevent this situation, the establishment of mineral-balanced pellets according to the needs of the produced fish is necessary to minimize mineral overload in aquatic habitats. Zinc (Zn) is also very important for appropriate growth rate, metabolism activity and immune system in fish [1,2]. Moreover, it has role in antibacterial actions, health-improving, and preventive outcomes in living animals [3] and is necessary for favourable somatic development [4]. On the other hand, zinc deficiency in fish causes problems in hormone system, growth (i.e.,

* Corresponding author: mharlioglu@firat.edu.tr. ORCID Number of authors: [1] 0000-0003-3290-1092, [2] 0000-0001-8288-0571, [3] 0000-0003-3735-6732, [4] 0000-0002-4393-3527.

reduces survival rates) and accelerates the formation of cataracts in the eye [5-7]. Considering their significant potential in the food industry and human health, antioxidants are gaining popularity all over the world. Antioxidants are defined as substances that can prevent or delay the oxidation of easily oxidized substances, even in small quantities. An antioxidant is also defined as a substance that can inhibit a certain oxidizing enzyme or react with oxidizing agents before damaging other molecules, or a substance that traps metal ions or even repairs the system, such as an iron carrier protein [8]. The living body contains enzymatic and non-enzymatic antioxidant systems such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). These systems govern the stability between antioxidants and reactive oxygen species (ROS) [9]. The major mechanism involved in the oxidative cell injury is lipid peroxidation which is a complex process of the breakdown of polyunsaturated fatty acids [10]. Malondialdehyde (MDA), the end product of peroxidation and oxidative damage caused by reactive oxygen species, has been reported to be a vital marker [11]. Antioxidants can protect living things from free radicals and the harmful effects of ROS and prevent the progression of lipid peroxidation [12]. Due to these effects, interest in natural additives with potential antioxidant contents is increasing [13]. Zinc is a necessary microelement demanded for the structure and function of enzymes and many macromolecules that regulate cellular processes. It exhibits antimicrobial, antioxidant, and anti-inflammatory activity by modulating the immune response. It delays oxidative processes for an extended duration by causing the definition of metallothionein [14]. It is an important nutrient that helps the normal development of the living thing and better development of the immune system [15]. Zinc conserves the membranes of cells from iron-commenced lipid oxidation by covering negatively debited areas with possible iron-binding power. However, the interdependent effects of zinc with water-soluble antioxidants and lipids prevent lipid oxidation [16]. In addition, zinc improves the mobilization of antioxidant proteins and enzymes (i.e., catalase and glutathione). It can also replace redox-active elements (i.e., iron and copper) at specific linking locations [14], and can decrease the inflammatory feedback of the body's branchial and viscera by enhancing the body's antioxidant and anti-stress protection abilities [17]. Furthermore, the potential of zinc for delaying oxidative manner is known for a long time. The antioxidant mechanism can be divided into acute and chronic. Although there are substantial verifications on the antioxidant effects of zinc, these processes have not yet been fully clarified. Future research investigating these processes can be possibly identified contemporary antioxidant properties and employs for zinc [18].

The purpose of the present investigation was to determine the antioxidant enzyme activities in the liver and muscle tissue of juvenile *C. carpio* fed diets containing different levels of zinc (T1, control, 85 mg Zn kg^{-1}, T2, 105 mg Zn kg^{-1}, T3, 125 mg Zn kg^{-1} and T4, 145 mg Zn kg^{-1}).

2. Materials and methods

2.1. Experimental protocol.

The Fırat University Ethics Committee's established rules for conducting experiments were followed for this study. In this study, a basal meal consisting of 4,300 kcal/kg gross energy, 6% crude fat, and 35 ± 0.02% crude protein was used (Table 1). The nutritional needs of juvenile common carp fish were taken into consideration when creating the criteria [19]. Then, the experimental meals (T1, control, 85 mg Zn kg^{-1}, T2, 105 mg Zn kg^{-1}, T3, 125 mg Zn kg^{-1} and T4, 145 mg Zn kg^{-1}) containing varied amounts of zinc sulphate monohydrate ($ZnSO_4 \bullet H_2O$, 35% Zn) were created. The proportions of the experimental meals and the control diets for dry matter are listed in (Table 1), crude protein, crude fat, gross energy (kcal/kg), crude ash, crude cellulose, lysin, methionine, calcium, and phosphorus. Also shown in (Table 2) are the percentages of fish meals, soybean meals, yellow maize, wheat flour, oil, vitamin and mineral mix in the control diet that was employed in this study.

In total, 240 juvenile carps (11.7 ± 0.4 g and 9 ± 03 cm in length) were used for this study. Carp juveniles were provided by The Government Water Management Affairs of IX. Area Directory, Keban, Elazığ, Türkiye. Three replicates were used in conducting this study. Each glass aquarium contained 20 juvenile carp (143 cm X 37 cm X 30 cm). The fish were exposed to the experimental conditions for two weeks to get used to the experimental environment. Throughout the period of acclimatization, fish were fed commercial feed. The experiment was set up in 158 L of water with 12 aquariums, one for each treatment, receiving 7 weeks of well-aerated, dechlorinated tap water. 240 juveniles (11.8 ± 0.1 g) were randomly assigned to 4 treatment groups, each with a replicate.

Table 1. The proximate composition of the basal diet in %.

Diet Items	%
Dry Matter	92.4
Crude protein	35
Crude Fat	6
Gross Energy (kcal/kg)	4.300
Raw Ash	7
Raw Cellulose	3.5
Lysine	1.9
Methionine	0.7
Calcium	1.5
Phosphorus	1.2
Zinc (mg/kg)	85

Table 2. Composition of the basal diet in %.

Ingredients	%
Fish Meal	20.0
Soybean Meal	35.0
Yellow Corn	21.5
Wheat flour	20
Fish oil	2.0
Vitamin Mix	1.0
Mineral Mix	0.5
Total	100

Vitamin Mix: Vitamin A 3.22 (I.U/100 g fillet), Vitamin D 208.40 (I.U/100 g fillet), Vitamin E 0.16 (I.U/100 g fillet), Vitamin K 0.16 (I.U/100 g fillet), and Vitamin C 1200 mg/kg. Minerla Mix mg/g: Fe 50, Cu 3, Co 0.01, Mn 20, I 0.1 and Se 0.1

The water was kept at a constant temperature of 27°C with a pH of 7-8, DO of 6 mg/L, total ammonia of 0.6 mg/L, and water Zn of 2.2 mg/L. The experiment was conducted as the sun went through its daily cycle, with each tank being continuously aerated. The amount of food that should be provided to the young each day was calculated using the formula below. It was administered twice daily in equal portions for 60 days.

The daily Feed Amount= Feeding Coefficient x Total Fish Weight / 100 considering water temperature [20]. Feeding coefficient values for 60 days were 2.058 for control, 1.431 for T1, 1.984 for T2 and 2.030 for T3. A commercial juvenile carp feed manufacturer (GÜRDAL YEM, Kahramanmaraş http://www.gurdalyem.com.tr/) provided the basic diet. "Kahramanmaraş Sütçü İmam Üniversitesi Üniversite-Sanayi-Kamu İşbirliği Geliştirme Uygulama ve Araştırma Merkezi (ÜSKM) Laboratuvarlar" conducted the content analysis of the experimental pellets.

The proportionate elements were mixed together until the dough was completely homogeneous, and then water was added in a 1/1 ratio to the dough. The pulp material was pelletized using the mincing equipment. The generated pellets were placed on trays and allowed to dry for 24 hours at 60°C in a feed oven. When determining the size of the pellets, consideration for the fish's weight was made. The rations were maintained in polypropylene storage containers to be used at 4°C.

2.2. Dissection and tissue preparation procedures

The bioengineering laboratories of Munzur University performed the dissection and processing of tissues for analysis. The carp that had been maintained in a deep freezer at -85°C for one day was removed and left to defrost for five hours in order to measure the enzyme activity in the tissues. The bioengineering laboratories of Munzur University performed tissue dissection and preparation for analysis. With sharp scissors, the fish's bellies were separated from the anus and cut all the way to the gills. A little piece of the liver, which is located right below the air sac, was excised, and weighed on a precise balance. Using a phosphate-buffered salt solution with a pH of 7.4 at a rate of 1/5 w/v, the blood was then extracted after the liver had been removed. The solution's pH was adjusted using diluted glucose. The homogenization process was followed by the placement of the liver pieces in Eppendorf tubes. To stop the enzymes from degrading as the homogenizer's cycle temperature increased, ice moulds were

utilized. The CAT underived homogenizer was used for homogenization. After homogenization, the tubes were cooled down and spin at 17000 rpm for 15 minutes in a Nuve 800 R centrifuge to produce supernatants.

2.3. Making analysis

Antioxidant kits were used to process the produced supernatants before being read by the microplate reader. Sunred kits were utilised for MDA and CAT, while BT-Lab kits were used for SOD activity. The kits were used for fish. Using automated micropipettes, samples from the supernatants were transferred to the 96 well plate that was included in the antioxidant kit box. The prepared plates were read in the microplate reader attached to the computer after following the instructions in the kit.

2.4. Statistical analysis

The SPSS 22.0 package programme was used to apply the ANOVA Duncan's test for the evaluation of this data collection. The letters "a, b" are used to represent the outcomes. The data distribution was subjected to a test.

3. Result

When the antioxidant analysis results were examined, it was determined that the SOD values of the liver were 0.623 ± 0.097 U/ml in the control group, decreased to 0.546 ± 0.033 U/ml in the T1 group, increased to 0.651 ± 0.084 U/ml in the T2 group, and attained the greatest value at 0.713 ± 0.220 U/ml in the T3 group. Although numerical differences were detected between the values, no statistically significant difference was found ($p > 0.05$). SOD values for the muscle showed a different situation than the liver, and while it was 0.607 ± 0.146 U/ml in the control group, it increased to 0.751 ± 0.144 U/ml in the T1 group. It started to decrease again with 0.690 ± 0.142 U/ml in the T2 group and 0.646 ± 0.170 U/ml in the T3 group ($p > 0.05$). Muscle and liver results did not show a parallel situation compared to the experimental groups (Table 3).

In the CAT results, while the value of the control group was 0.876 ± 0.067, this value decreased to 0.818 ± 0.117 nmol/dk/m in the T1 group, 0.849 ± 0.115 nmol/dk/m in the T2 group and suddenly decreased to 0.728 ± 0.127 nmol/dk/m in the T3 group ($p < 0.05$). When examined for muscle, the CAT value was 0.724 ± 0.157 nmol/dk/m in the control group, increased to 0.874 ± 0.184 nmol/dk/m in the T1 group, reached the highest value with 1.059 ± 0.148 nmol/dk/m in the T2 group, and 0.854 ± 0.142 nmol/dk/m with a sudden decrease in the T3 group ($p < 0.05$). Muscle and liver results did not show a parallel situation compared to the experimental groups (Table 3).

In the liver analysis of malondialdehyde (MDA), the value in the control group was 1.866 ± 0.255 µM, with a slight increase in the T1 group, it became 1.888 ± 0.419 µM, then suddenly decreased to 1.671 ± 0.230 µM in the T2 group and again reached a value close to 1.868 ± 0.567 µM in the T3 group. ($p > 0.05$). When the muscle data were examined, it was 1.313 ± 0.124 µM in the control group, while it increased to 1.428 ± 0.238 µM in the T1 group, with a slight increase in the T2 group, a value of 1.498 ± 0.207 µM was obtained, and then again with a rapid increase, it reached the highest value of 1.620 ± 0.124 µM ($p < 0.05$). Muscle and liver results did not show a parallel situation compared to the experimental groups (Table 3).

Table 3. SOD, CAT and MDA values (average ± SD) in liver and muscle tissues of carp fish.

	Control (T1)	T2	T3	T4
	SOD values (average ± SD) in liver and muscle tissues of carp fish) (U/ml)			
Liver	0.623±0.097[a]	0.546±0.033[a]	0.651±0.084[a]	0.713±0.220[a]
Muscle	0.607±0.146[a]	0.751±0.144[a]	0.690±0.142[a]	0.646±0.170[a]
	CAT values (average ± SD) in liver and muscle tissues of carp fish (nmol/dk/m)			
Liver	0.876±0.067[a]	0.818±0.117[ab]	0.849±0.115[ab]	0.728±0.127[b]
Muscle	0.724±0.157[a]	0.874±0.184[ab]	1.059±0.148[b]	0.854±0.142[a]
	MDA values (average ± SD) in liver and muscle tissues of carp fish) (µM)			
Liver	1.866±0.255[a]	1.888±0.419[a]	1.671±0.230[a]	1.868±0.567[a]
Muscle	1.313±0.124[a]	1.428±0.238[ab]	1.620±0.124[b]	1.498±0.207[ab]

4. Discussion

Humans can easily absorb and digest important micro and macro elements and proteins in fish meat [21]. On the other hand, it is a challenge for public health to deal with Zn water contamination by industrial expansion [22]. It has been found that Zn accumulates greater amounts in fish feces, creating critical problems in humans [23]. In addition, Zn storing gives rise to nourishing diseases and oxidative stress in animals [24]. For this reason, it is important to develop water treatment technologies to eliminate Zn toxicity [25] as shown in recent studies.

Like other vertebrates, fish have an antioxidant system to deal with oxidation and oxidation reactions occur in the organism through enzymes containing metals such as iron (Fe), copper (Cu) and Zn [26]. In order to alleviate the damage caused by oxidative stress, antioxidant molecules containing glutathione (GSH) undertake the first line of defence [27]. Secondary defence systems consist of antioxidant enzymes such as SOD, which detoxifies superoxide anions, and CAT radical scavenger, which reduces H_2O_2 [27,28]. Dawood et al. [29] indicated that feeding rabbitfish with dietary ZnMet (30 mg/kg) for 49 days showed increased protein utilization, development rate, CAT, SOD, GPx, phenol oxidase activities, and lysozyme. It is also known that MDA is a good biomarker to measure oxidative stress [30]. Lipid peroxidation is the oxidative degradation process of lipids, in which free radicals cause cell damage, especially by taking electrons from polyunsaturated fatty acids (PUFA) in cell membranes [31]. It was found that supplementing diets with excellent levels of zinc extremely improves the antioxidant actions of fish [32,33].

Ibrahim et al. [34] found that growth and the immune system improved, and the amount of growth and antioxidant enzymes (SOD and CAT) increased, at the end of the study in which they fed a fish with antioxidant feed. Furthermore, as a result of a study conducted to evaluate the antioxidant effect of dietary Zn levels on juvenile yellow catfish (*Pelteobagrus fulvidraco*), a significant decrease in MDA level was observed [33], In addition, it was observed that SOD activity rose and malondialdehyde (MDA) amount decreased with the increase in nutritional Zn amounts reached the required level. Hepatic CAT activity did not differ significantly between treatments. The SOD and CAT values of the finding obtained in this investigation are unchanging with the above studies. Only the muscle values of the MDA analysis were quite different from these studies, and the liver values were not very different from the control group, even if the liver values were not as low as desired. The best value for MDA was obtained in the T2 muscle trial group.

Excessive absorption of Zn adversely affects reproductive performance in fish and may limit the uptake and use of other minerals [34]. Furthermore, it can disrupt the metabolic activities that cause both ion balance and oxidative destruction in fish [35,36]. As a result of ZnO toxicity studies in fish, an average dose value was found [37,38]. Depending on this situation, it is recommended to use ZnONPs in average amounts to diminish the harmful effects on the toxicity range and development rate [39,40].

Wu et al. [41] mentioned that fed young grass carp (*Ctenopharyngodon idella* Val.) with Zn-added feeds. The weight gain, specific growth rate, feed intake, feed conversion rate, SOD and CAT activities increased significantly up to a point and then decreased with increasing Zn levels. Malondialdehyde (MDA), on the other hand, decreased significantly with increasing zinc level up to a point and then increased again. Although muscle values in the results obtained from SOD analyses revealed values similar to the above study, liver results on the contrary showed a continuous increase. CAT analysis studies, whereas, revealed values more compatible with the above study. MDA values, on the other hand, gave more similar results to the above study.

Musharraf [42] indicated that in the study on the nutritional zinc demand for Indian major carp, serum superoxide dismutase, GPX, CAT, and alkaline phosphatase actions returned certainly, at the same time as malondialdehyde amount was 51.42 mg kg^{-1} nutritional zinc gave a negative response. Similar results were obtained in this study as well. While the recommended Zn ratio in fish for Zn is 35 mg kg^{-1}, the use of much higher amounts of Zn in the above study and in the feed used in this study may be the reason why the results are similar.

Huang et al. [7] conducted a study to observe the nutritional Zn demand of adult Nile tilapia and to assess its impacts on antioxidant responses. The weight gain (%) of the fish developed with accelerating nutritional Zn from 15.9 to 53.5 mg/kg for more than a period of 84 days and then fell over these amounts. It revealed that the activities of total superoxide dismutase content increased significantly, while catalase activities reduced remarkably with the expansion of nutritional Zn grade.

Feng et al. [43] studied the influences of nutritional Zn on lipid peroxidation, amino acid oxidation, and antioxidant defence of juvenile Jian carp by nourishing them with increased Zn amounts. The findings indicated that the content of malondialdehyde (MDA) in the serum was lowest in the food having 15.3 mg zinc kg^{-1}. SOD and CAT were increased with escalating nutritional Zn up to 40.8 mg zinc kg^{-1} food and then stabilized. The findings of the present investigation showed that ZN reduces lipid peroxidation and amino acid oxidation and improves antioxidant defence in carp juveniles. Although it could not reach the desired values, Zn showed positive effects on antioxidant values SOD and CAT and MDA values in this research.

Saddick et al. [44] informed that the lethal concentrations (LC50) of Zinc nanoparticles (ZnNPs (500 and 2000 μg L^{-1})) on *Oreochromis niloticus* and *Tilapia zillii*. As a result of the study, while SOD and CAT values decreased, MDA values increased significantly. The reason why the analysis results are not completely perfect in Zn feeding may be due to the fact that the Zn ratio is slightly higher, as we have mentioned before.

Mohammadya et al. [45] found that with higher Zn availability than the ZnSO4 form, it promoted growth, modulated digestive enzymes, improved serum biochemical response and immune antioxidant enzyme capacity in Nile tilapia. In addition, they found that iinsufficient zinc consumption in a short time may show harmful symptoms and its effect may become evident. However, the bone mineralization and growth performance of fish might be badly affected by using the huge level of Zn consumption [34], Moreover, [34], stated that high zinc consumption may negatively affect the bone mineralization and growth performance of fish.

Kumar et al.; Alvarez et al. [46,47], studied the influence of Zn on the development rate and cellular metabolic stress of fish maintained to various stresses affected by the high temperature due to climate change. Yu et al. [48] at the end of research indicated that where they fed *Oncorhynchus kisutch* puppies with Zn-added feeds, found that as the amount of Zn in the feeds increased. The results showed that the feed conversion ratio (FCR) was improved in the Pb and high temperature–maintained fish. On the other hand, zinc supplementation in the carp fish diet has enhanced weight gain (%), FCR, PER, and SGR. Compared to the study of [46,49], the results obtained in our study are better, especially in feed conversion ratio.

5. Conclusion

Based on the results of the present research, it is possible to significantly improve antioxidant defense by using convenient level of zinc in carp fish juvenile diet (105 mg/kg). Especially the results of SOD and CAT analysis confirm the positive effects of using 105 mg Zn in the diets to increase the growth performance of juvenile carp in this study. Further researches are required to investigate the zinc demands of different juvenile size of carps. In addition, the differences in dietary Zn demands among fish species can be due to the adaptive mechanism of metal absorption and utilization in their habitats when the results obtained in this study are compared with the results of other studies in the literature. Therefore, further research is needed on the characteristics and structures of the developmental adaptation of fish to mineral demand. The findings suggest that supplementing the diet of common carp fry with appropriate levels of zinc can have positive effects on antioxidant enzyme activities in their muscle and liver tissues. This indicates that zinc plays a significant role in the nutrition and overall health of carp fish. However, it is important to avoid excessive zinc supplementation, as it may lead to adverse effects on fish health and growth.

References

[1] National Research Council (NRC). Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC 2011; (376 + XVI p).
[2] Moazenzadeh K, Islami HR, Zamini A, Soltani M. Dietary zinc requirement of Siberian sturgeon (*Acipenser baerii*, Brandt 1869) juveniles, based on the growth performance and blood parameters. Int Aquat Res 2017; 9: 25-35.
[3] Wang B, Feng W, Wang M, Wang T, Gu Y, Zhu M. Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. J Nanopart Res 2008; 10(2): 263-276.
[4] Paski SC, Xu Z. Labile intracellular zinc is associated with 3T3 cell growth. J.Nutr Biochem 2001; 12(11): 655-661.
[5] Clegg MS, Keen CL, Donovan SM. Zinc deficiency induced anorexia influences the distribution of serum insulin-like growth factor binding proteins in the rat. Metab 1995; 44(11): 1495-1501.
[6] Ekinci D, Ceyhun SB, Aksakal E, Erdoğan O. IGF and GH mRNA levels are suppressed upon exposure to micromolar concentrations of cobalt and zinc in rainbow trout white muscle. Comp Biochem Physiology Part - C Toxicol Pharmacol 2011; 153(3): 336-341.
[7] Huang F, Jiang M, Wen H, Wu F, Liu W, Tian J, Yang C. Dietary zinc requirement of adult Nile tilapia (*Oreochromis niloticus*) fed semi-purified diets, and effects on tissue mineral composition and antioxidant responses. Aquacult 2015; 439: 53-59.
[8] Brewer MS. Natural Antioxidants: Sources, Compounds, mechanisms of action, and potential applications. J Food Sci Educ 2011; 10(4): 221-247.
[9] Salehi B, Martorell M, Arbiser JL, Sureda A, Martins N, Maurya PK, Sharifi-Rad M, Kumar P, Sharifi-Rad J. Antioxidants: positive or negative actors? Biomol 2018; 8(4): 124.
[10] Meriç, İ. Evaluation of sunflower seed meal in feeds for carp: Antinutritional effects on antioxidant defense system, JFAE 2013: 11(2): 1128-1132.
[11] Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin Chem 1997; 43: 1209-1214.
[12] Kalaiselvi T, Panneerselvam C. Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. JNB 1998; 9: 575-581.

[13] Ma JJ, Xu ZR, Shao QJ, Xu JZ, Hung Silas SO, Hu WL, Zhuo LY. Effect of dietary supplemental L-carnitine on growth performance, body composition and antioxidant status in juvenile black sea beram, *Sparus macrocephalus*. Aquac Nutr 2008; 14: 464-471.
[14] Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc zinc-dependent NF-κB signalling. Inflammopharma 2017; 25: 11-24.
[15] Fazil DM, Hamdi H, Al-Barty A, Zaid AA, Parashar SKS, Das B. Selenium and zinc oxide multinutrient supplementation enhanced growth performance in zebra fish by modulating oxidative stress and growth-related gene expression. Front Bioeng Biotechnol 2021; 9: 1-12.
[16] Zagoa MP., Oteiza PI. The antioxidant properties of zinc: interactions with iron and antioxidants. Free Radic Biol Med 2001; 31(2): 266-274.
[17] Ren HT, An HY, Du MX, Zhou J. Effects of zinc adaptation on histological morphology, antioxidant responses, and expression of immune-related genes of grass carp (*Ctenopharyngodon idella*). Biol Trace Elem Res 2022; 200(12): 5251-5259.
[18] Powell SR. The antioxidant properties of Zinc. J Nutr 2000; 130(5): 1447-1454.
[19] National Research Council (NRC) (1993). Nutrient Requirements of Fish, Washington, D.C, USA, National Academies Press. 114 p.
[20] Song-bo CH, Wei-xing CH, Zhao-ting F. Effect of water temperature on feeding rhythm in common carp (*Cyprinus carpio*) haematopterus Temminck Schlegel. J Northeast For Univ 2012; 19(1): 57-61.
[21] Wang N, Yin Y, Xia C, Li Y, Liu J, Li Y. Zn-enriched *Bacillus cereus* alleviates Cd toxicity in mirror carp (*Cyprinus carpio*): Intestinal microbiota, bioaccumulation, and oxidative stress. Biol Trace Elem Res 2021; 200: 1-10.
[22] Liu F, Li M, Lu J, La, Z, Tong Y, Wang M. Trace metals (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) and stable isotope ratios (δ13C and δ15N) in Fish from Wulungu Lake, Xinjiang, China. IJERPH 2021; 18: 9007.
[23] El-Moselhy KM, Othman A, Abd El-Azem H, El-Metwally M. Bioaccumulation of heavy metals in some tissues of fish in the Red Sea. Egypt J Basic Appl Sci 2014; 1: 97-105.
[24] Connolly M, Fernández M, Conde E, Torrent F, Navas JM, Fernández-Cruz ML.Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout. Sci Total Environ 2016; 551: 334-343.
[25] Loro VL, Jorge MB, da Silva KR, Wood CM. Oxidative stress parameters and antioxidant response to sublethal waterborne zinc in a euryhaline teleost *Fundulus heteroclitus*: protective effects of salinity. Aquat Toxicol 2012; 110: 187-193.
[26] Yapici M, Meriç Turgut İ. Farklı doz ve sürelerde levonorgestrel uygulamasının zebra balıklarında (D*anio rerio*, Hamılton, 1822) antioksidatif metabolizma üzerine etkileri, Ankara Üniversitesi Fen Bilimleri Enstitüsü, 2023, Ankara. PhD thesis, 80 p.
[27] Roberts AP, Oris JT. Multiple biomarker response in rainbow trout during exposure to hexavalent chromium. Comp Biochem Physiol Part - C Toxicol Pharmacol 2004; 138: 221-228.
[28] Bagnyukova V, Chahrak OI, Lushchak V. Coordinated response of goldfish antioxidant defences to environmental stress. Aquat Toxicol 2006; 78: 325-331.
[29] Dawood MAO, Alagawany M, Sewilam H. The role of zinc microelement in aquaculture: a Review. Biol Trace Elem Res 2021; 200(8): 3841-3853.
[30] Wang J, Xiao J, Zhang J, Chen H, Li D, Li L, Cao J, Xie L, Luo Y. Effects of dietary Cu and Zn on the accumulation, oxidative stress and the expressions of immune-related genes in the livers of Nile tilapia (*Oreochromis niloticus*). Fish Shellfish Immunol 2020; 100: 198-207.
[31] Kanazawa K. Tissue injury induced by dietary products of lipid peroxidation. In: Corongiu, F. (Ed.), Free Radicals and Antioxidants in Nutrition. Richelieu Press, London, 1993; 383-399.
[32] Jiang N, Wu F, Huan F, Wen H, Liu W, Tian J, Yang C, Wang W. Effects of dietary Zn on growth performance, antioxidant responses, and sperm motility of adult blunt snout bream, *Megalobrama amblycephala*. Aquacult 2016; 464: 121-128.
[33] Luo Z, Tan XY, Zheng JL, Chen QL, Liu CX. Quantitative dietary zinc requirement of juvenile yellow catfish *Pelteobagrus fulvidraco*, and effects on hepatic intermediary metabolism and antioxidant responses. Aquacult 2011; 319(1-2): 150-155.
[34] Ibrahim MS, Mohammady EY, El-Erian MA, Ragaza JA, El-Haroun ER, Hassaan MS. Dietary zinc oxide for growth and immune stimulation of aquatic animal species: A Review. Proc Zool Soc 2023; 76: 59-72.
[35] Shiau SY, Jiang C. Dietary zinc requirements of grass shrimp, *Penaeus monodon*, and effects on immune responses. Aquacult 2006; 254: 476-482.
[36] Shyong JS, Sun LT. Effects of dietary zinc levels on zinc concentrations in tissues of common carp. J Nutr 1981; 111: 134-140.
[37] Wehmas LC, Anders C, Chess J, Punnoose A, Pereira CB, Greenwood JA. Comparative metal oxide nanoparticle toxicity using embryonic zebrafish. Toxicol Rep 2015; 2: 702-715.
[38] Verma SK, Mishra AK, Suar M, Parashar SKS. In vivo assessment of impact of titanium oxide nanoparticle on zebrafish embryo. AIP Conference Proceedings 2017; 1832, 040030.
[39] Franklin NM, Rogers NJ, Apte SC, Batley GE, Gadd GE, Case PS. Comparative toxicity of nanoparticulate ZnO, bulk ZnO, and ZnCl2to a freshwater microalga (*Pseudokirchneriella subcapitata*): The importance of particle solubility. ESST 2007; 41: 8484-8490.
[40] Lin D, Xing B. Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Envir Pollut 2007;150: 243-250.

[41] Wu YP, Feng L, Jiang WD, Liu Y, Jiang J, Li SH, Tang L, Kuang SY, Zhou XQ. Influence of dietary zinc on muscle composition, flesh quality and muscle antioxidant status of young grass carp (*Ctenopharyngodon idella* Val.). Aquac Rep 2015; 45(10): 2360-2373.
[42] Musharraf M, Khan MA. Dietary zinc requirement of fingerling Indian major carp, *Labeo rohita* (Hamilton). Aquac Rep 2019; 503: 489-498.
[43] Feng F, Tan LN, Liu Y, Jiang J, Jiang WD, Hu K, Li SH, Zhou XQ. Influence of dietary zinc on lipid peroxidation, protein oxidation and antioxidant defence of juvenile Jian carp (*Cyprinus carpio* var. Jian). Aquac Nutr 2011; 17(4): 875-882.
[44] Saddick S, Afifi M, Abu Zinada OA. Effect of zinc nanoparticles on oxidative stress-related genes and antioxidant enzymes activity in the brain of *Oreochromis niloticus* and *Tilapia zillii*. Saudi J Biol Sci 2017; 24(7): 1672-1678.
[45] Mohammadya EY, Soaudyb MR, Abdel-Rahmanc A, Abdel-Tawwabd M, Hassaan MS. Comparative effects of dietary zinc forms on performance, immunity and oxidative stress-related gene expression in Nile tilapia, *Oreochromis niloticus*. Aquacult 2021; 532: 1-11.
[46] Kumar N, Krishnani KK, Singh NP. Effect of zinc on growth performance and cellular metabolic stress of fish exposed to multiple stresses. Fish Physiol Biochem 2020; 46: 315-329.
[47] Alvarez RM, Morales AE, Sanz A. Antioxidant defences in fish: biotic and abiotic factors. Rev Fish Biol Fish 2005; 15: 75-88.
[48] Yu, HR, Li LY, Shan LL, Gao J., Ma CY, Li X. Effect of supplemental dietary zinc on the growth, body composition and anti-oxidant enzymes of coho salmon (*Oncorhynchus kisutch*). Aquac Rep 2021; 20: 100744.
[49] Avila-Nava A, Pech-Aguilar AG, Lugo R, Medina-Vera I, Guevara-Cruz M, Gutiérrez-Solis A. Oxidative stress biomarkers and their association with mortality among patients infected with SARS-CoV-2 in Mexico. Oxidative Med Cell Longev 2022: 1-8.