Impact of Iron Oxide and Zinc Oxide Nanoparticles Supplementation on some Biochemical and Hematological Parameters in Cyprinus Carpio L.

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Abstract

This study focused on evaluating the effect of using iron oxide and zinc oxide nanoparticles on some of biochemical and blood parameters of Cyprinus Carpio L. carp. The study evaluated red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV), various metabolic compounds, and liver enzymes activity. The experimental fish were fed seven diet treatments, each treatment containing a different concentration of nanomaterials (iron oxide nanoparticles with a size ranging between (20-40 nm) and zinc oxide nanoparticles with a size ranging between (35-45 nm), the first treatment T1 control, the second treatment 30 mg/kg, and the fourth 40 mg/kg of nano iron oxide, the third treatment 30 mg/kg, and the fifth 40 mg/kg of nano zinc oxide, the sixth treatment 30+30 mg/kg, and the seventh 40+40 mg/kg of iron oxide and zinc oxide nanoparticles respectively. The results indicated that the second and fifth nanoparticle treatments significantly improved blood laboratory test outcomes ($P<0.01$), with increases in packed cell volume (PCV) and hemoglobin levels, while white blood cell and red blood cell counts decreased. Biochemically, total protein and glucose levels increased, while liver enzyme levels decreased. However, cholesterol and triglyceride levels remained unaffected. In conclusion, Incorporation of nanoparticles into carp feed provides many benefits, including increased nutrient absorption, increased metabolism, improved growth rates, and improved overall fish health by acting as antioxidant .

Keywords: Common carp, nano zinc oxide, nano iron oxide .

Introduction:

Fish are an important source of protein and essential amino acids globally and locally and are considered a source of food, as aquatic organisms constitute 17% of the protein consumed in the world [8]. Studying the chemical composition of fish is an important aspect, as it can be used to determine their nutritional value. The protein content of fish bodies has been studied and analyzed, and the protein ratios ranged between 15-20%, including all the essential amino acids that meet the body's needs [2]. The demand for fish is increasing globally as a primary source of protein, prompting farmers to increase their farming rates. This increase in fish farming requires large quantities of feed to bridge the gap in feed prices. [9] Traditional fish feeds consist of macronutrients (proteins, fats, and carbohydrates), micronutrients (vitamins and minerals), and other essential components obtained mainly from marine products such as fishmeal and fish oil [16] Nutrition studies have focused on improving feed quality using various growth promoters such as antibiotics as feed additives. However, after the ban on the use of antibiotics as growth promoters in fish feeds, new strategies in nutrition and

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health management in aquaculture systems have gained much attention using new technologies such as nanotechnology to develop and improve feed quality [14] Nanomaterials are those with dimensions ranging from 1 to 100 nm, and as a result, their properties differ from those of non-nano materials in their natural state [4]. Nanometallics have several properties including high surface area affinity as well as high solubility, which makes them ideal for developing new technologies to enhance the health of aquatic organisms including common carp Cyprinus carpio L. by improving their production performance [6]. Iron and zinc are rare and important minerals in fish nutrition, as they play a major role in the metabolism process. Iron is also a component of hemoglobin, which transports oxygen, and zinc is involved in tissue building and growth and regeneration of immune cells [5]. This study aims to evaluate the effect of adding zinc oxide and iron oxide nanomaterials to the diet of common carp (Cyprinus carpio) on their blood and biochemical parameters .

Materials and Methods

The current study shows the effect of adding different levels of nano-iron oxide and nano-zinc oxide to common carp diets on some hematological and biochemical parameters in the blood of the experimental fish. This experiment used 182 common carp fish, weighing between 5-10 grams, and they were distributed into 7 treatments, each treatment containing 26 fish. The duration of the experiment was 70 days. The first treatment was a control (T1) free of additives, the second treatment contained 30 mg/kg nano-iron oxide, the third treatment contained 30 mg/kg nano-zinc oxide, the fourth treatment contained 40 mg/kg nano-iron oxide, the fifth treatment contained 40 mg/kg nano-zinc oxide, the sixth treatment contained 30+30 mg/kg nano-iron oxide and nano-zinc oxide, and the seventh treatment contained 40+40 mg/kg nano-iron oxide and nano-zinc oxide. At the end of the experiment, the injection needle or syringe was prepared by placing heparin powder inside the syringe to prevent blood clotting and coagulation. Then, blood samples were drawn directly from the heart using a 2 ml medical syringe. Then, the samples were divided into two parts: the first part in EDTA tubes for the purpose of conducting blood count for fish, which include RBC, white blood cells (WBC), (PCV), and Hb based on the method mentioned by [15]. The other part of the blood was transferred to gel tubes and separated by a centrifuge to obtain the serum. After that, the samples were placed after numbering in a refrigerated container to preserve the samples and they were transferred to the laboratory and frozen until the analysis was conducted .

For total protein, glucose, triglyceride, GOT, and GPT analysis, Humman Kits (Germany) where used to calculate their concentrations in the collected blood .

Statistical Analysis

In the statistical analysis, a completely randomized design (CRD) was used to study most of the productive traits of common carp fish cultured in rearing ponds using the Statistical Package for the Social Sciences (SPSS), version 26, and to extract significant differences between the averages of the studied traits using Duncan's multi-range test [7] at levels Significant (0.05.(

Result and Discussion

Hematological parameters

Blood parameters are considered one of the most important indicators in determining the health and well-being of fish. These criteria

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include: Red blood cell count, white blood cell count, hemoglobin, and packed cell volume.

White blood cell count (WBC:(

The results of the statistical analysis presented in Table 1 for the blood parameters, specifically white blood cell (WBC) count, showed a decrease in numbers for the fifth treatment (T5), fourth treatment (T4), and second treatment (T2) with values of 5.6, 5.95, and 6.0, respectively, compared to the control treatment (T1) with a value of 7.35. White blood cells are a crucial part of the immune system in fish, serving as the first line of defense against pathogens. The number of WBCs is highly influenced by oxidative stress, which is why nanomaterials, acting as antioxidants, can support the immune system. This suggests that nanoparticles may reduce the need for antibiotics and help minimize the risk of disease outbreaks.

Red blood cell counts (RBC:(

Table (1) indicated that red blood cell count showed a positive effect of nanoparticles added to carp fish feed in the different experimental treatments, as a decrease in the level of red blood cell count was observed in all treatments compared to the control treatment. This decrease indicates that nanoparticles had an important impact on the health of the circulatory system in fish. The fifth treatment T5 outperformed all treatments, and the sixth treatment T6, and the seventh treatment T7 were significantly similar to it, followed by the third treatment T3, while the second and fourth treatments were the least superior among the experimental treatments.

The results of the current study showed that the addition of nano-iron oxide and nano-zinc oxide had no positive effect on red blood cells and white blood cells. These results were consistent with the previous study by researchers [13] who stated that fish injected with water mixed with nanomaterials at limited concentrations experienced a decrease in the levels of white blood cells and red blood cells within the normal level.

Packed cell volume (PCV) and hemoglobin (Hb:(

It was observed through statistical analysis in Table (1) the effect of nano-iron oxide and nano-zinc oxide, which were mixed with a common carp fish diet, on PCV and hemoglobin Hb, as the second treatment, T2 (30 mg/kg of nano-iron oxide), was significantly superior to all experimental treatments. This transaction is significantly similar to the fourth transaction T4 and the third transaction T3, followed by the seventh transaction T7. The sixth is T6, and the least superior is the fifth treatment, T5.

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Table 1. Effect of adding nano iron oxide and nano zinc oxide to fish feed on blood parameters in common carp.

Treatments	Weight average ± standard error			
	PCV (%)	Hb g/ dl	RBC (million / µl)	WBC × 10^3 /ml^3
T1	8.30 b ± 0.20	2.75 b ± 0.05	3.50 a ± 0.20	7.35 a ± 0.25
T2	12.66 a ± 0.54	4.21 a ± 0.11	3.00 ab ± 0.20	6.00 b ± 0.30
T3	11.11 a ± 0.10	3.66 a ± 0.0	2.80 bc ± 0.0	7.60 a ± 0.0
T4	12.03 a ± 0.99	4.00 a ± 0.33	3.00 ab ± 0.20	5.95 b ± 0.25
T5	8.11 b ± 0.0	2.66 b ± 0.0	2.20 c ± 0.10	5.60 b ± 0.50
T6	8.58 b ± 0.54	2.83 b ± 0.17	2.30 c ± 0.20	6.25 b ± 0.15
T7	8.86 b ± 0.35	2.84 b ± 0.18	2.30 c ± 0.20	6.25 b ± 0.15
significantly	**	**	**	**

T1= control treatment, T2= addition of 30 mg/kg nano iron oxide, T3= addition of 30 mg/kg nano zinc oxide, T4= addition of 40 mg/kg nano iron oxide, T5= addition of 40 mg/kg nano zinc oxide, T6= addition of 30+30 mg/kg each of nano iron oxide and nano zinc oxide, T7= addition of 40+40 mg/kg each of nano iron oxide and nano zinc oxide .

**Different letters within the same column mean significant differences between the means of the treatments at the level (P<0.01)., N.S.: Not Significant

Through the data of table (1), the effect of adding nano-iron oxide and nano-zinc oxide to the common carp feed on the blood parameters (hemoglobin Hb and PCV) was observed, and the second treatment (T2) was significantly superior, followed by the fourth treatment (T4) and then the third treatment (T3). This experiment proved that the use of nano-iron oxide and nano-zinc at a concentration of 30 mg/kg and 40 mg/kg reduces the number of white and red blood cells and increases the level of hemoglobin and the packed cell volume (PCV) in the blood of common carp. These parameters are important indicators in determining the health and well-being of living organisms. These indicators also work to transport oxygen from the gills to all parts of the body, enhance the absorption of iron in the body of the living organism, and improve

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the metabolic process. These substances also work as antioxidants. These results obtained from the current study are consistent with many recent research and studies, including [1] which addressed Adding nano-iron oxide and nano-copper to fish feed improved growth and nutrition indicators and some blood parameters such as red blood cell, hematocrit, and hemoglobin compared to treatments fed on feeds containing iron and copper in their natural, non-nano form.

Biochemical standards

Biochemical parameters are one of the important indicators in determining the health status of fish. Biochemical tests include Glucose, Total Protein, Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT(

Total protein and glucose along with liver enzymes (GOT, and GPT

The results of the statistical analysis in Table (2) showed the effect of adding nano-iron oxide and nano-zinc oxide to the common carp feed on the biochemical characteristics (glucose). A clear significant superiority ($P<0.01$) was observed for the fifth treatment (40 mg/kg nano-zinc oxide) over all different experimental treatments, while this treatment was significantly similar to the second treatment (30 mg/kg nano-iron oxide), followed by the third, seventh, fourth, first and last treatments (sixth). All these treatments were at the same level and there was no significant difference ($P<0.01$) between them.

A high glucose level is considered a positive indicator and an energy source that facilitates the use of metabolism, digestion, and absorption. Glucose is also an important source of energy that the body needs to perform daily activities. It is one of the important biochemical criteria in fish, as it acts as a basic substrate for stored energy in the body that can be used when needed, especially in cases of stress or metabolic demand.

The hormones insulin and cortisol regulate blood glucose levels to meet the body's energy needs without causing blood sugar to spike. The results of the current study are consistent with the previous study by [11[

The second treatment, T2, and the fifth, T5, also outperformed the control treatment, T1, as they showed an increase in the level of total protein within the normal limits determined by the [3] this increase along with a decrease in the level of liver enzymes within the permissible limits that were indicated. By [10] in various treatments fed with nanomaterials is an indicator of the health of the liver and the absence of accumulation of nanomaterials in it.

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Table 2. The effect of adding nano-iron oxide and nano-zinc oxide to the common carp fish feed on the biochemical characteristics (total protein, glucose, and liver enzymes) in the blood serum of the experimental fish.

Treatments	weight average ± standard error			
	GLOGUSE mg/dl	PROTIEN g/dl	GPT U/L	GOT U/L
T1	130.50 b ± 4.50	3.10 ± 0.0	30.50 ± 0.50	26.00 ± 1.00
T2	160.0 a ± 7.00	3.40 ± 0.30	31.50 ± 0.50	27.50 ± 2.50
T3	140.0 b ± 0.0	2.70 ± 0.0	29.00 ± 0.0	28.00 ± 0.0
T4	130.50 b ± 4.50	2.90 ± 0.20	31.50 ± 0.50	27.50 ± 2.50
T5	160.50 a ± 1.50	3.30 0.10	30.50 ± 0.50	28.00 ± 1.00
T6	129.0 b ± 3.00	2.95 ± 0.25	30.50 ± 1.50	29.00 ± 1.00
T7	137.50 b ± 2.50	2.90 ± 0.20	30.00 ± 1.00	26.50 ± 1.50
significantly	**	N.S.	N.S.	N.S.

Cholesterol and triglyceride levels in common carp blood

The results of the statistical analysis data in Table (3) showed that there were no significant differences between the different experimental treatments in cholesterol and triglycerides levels and that the results of the experiment fall within the safe ranges registered with the [12] and This is a good indicator and an additional point for nanomaterials and can be used to assess the nutritional status and heart health of fish .

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Table 3. The effect of adding nano-iron oxide and nano-zinc oxide to the common carp fish feed on the biochemical characteristics (cholesterol and triglyceride levels) in the blood serum of the experimental fish.

Treatments	weight average ± standard error				
	VLDL mg/dl	**LDL mg/dl**	**HDL mg/dl**	**Triglyceride mg/dl**	**Cholesterol mg/dl**
T1	59.00 ± 2.00	111.50 ± 6.50	31.50 ± 1.50	246.5 ± 1.5	159.5 ± 4.50
T2	59.00 ± 2.0	111.50 ± 6.50	31.50 ± 1.50	246.5 ± 1.5	159.5 ± 2.5
T3	50.00 ± 4.00	95.00 ± 7.00	27.00 ± 2.00	207.50 ± 13.50	136.50 ± 9.50
T4	57.00 ± 0.0	105.00 ± 0.0	30.00 ± 0.0	274.50 ± 26.50	150.00 ± 0.0
T5	53.50 ± 3.50	100.50 ± 4.50	28.50 ± 1.50	283.00 ± 18.00	144.00 ± 6.00
T6	55.50 ± 5.50	105.50 ± 9.50	30.00 ± 3.00	243.00 ± 22.00	151.50 ± 13.50
T7	59.00 ± 2.00	110.00 ± 5.00	31.50 ± 1.50	261.00 ± 40.00	157.50 ± 7.50
significantly	**N.S.**	**N.S.**	**N.S.**	**N.S.**	**N.S.**

Conclusion

Adding nano iron oxide and nano zinc oxide to common carp feed at specific concentrations improves blood values and indicators in fish, reduces the rate of white blood cells, which indicates the functioning of the immune system without any stress, reduces the number of red blood cells, which is also an important criterion to know that the fish are in a state of well-being and that the oxygen level reaches an ideal level, while the increase in hemoglobin and compacted cells is also a positive factor and an important indicator to know that the nanoparticles have had a positive effect.

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