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Effect of Iron Oxide Nanoparticles and Zinc Oxide Nanoparticles as Nutritional Additives on Growth Performance and Feed Efficiency in *Cyprinus carpio* L.

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Abstract: This study aimed to evaluate the effect of adding different levels of Fe_2O_3 and ZnO nanoparticles to the diet of common carp fingerlings on their growth parameters, feed efficiency, and physical properties. A total of 182 fingerlings of common carp fish were used with uniform sizes and weight ranging from 7 to 10 g, regardless of sex. For 70 days, fish were fed with a diet containing 30% crude protein, to which nanoparticles were added at different levels. This experiment was conducted with seven treatments, including a control group with two replicates for each. Iron oxide nanoparticles and zinc oxide nanoparticles were added at varying concentrations to each treatment. Morphological characteristics were recorded for each replicate in each treatment every two weeks. The results demonstrated a significant improvement in weight gain, growth rate, and overall performance in all treatments compared to the control group. Specifically, the treatments fed with Fe_2O_3-NPs at 30 mg/kg (T2) and ZnO-NPs at 40 mg/kg (T5) showed highly significant improvements ($P<0.01$) In conclusion, both Fe_2O_3-NPs and ZnO-NPs effectively enhance growth performance and feed efficiency in *Cyprinus carpio*. These nanoparticles significantly improve weight gain and feed conversion, suggesting their potential to optimize growth and feed utilization in aquaculture.

Keywords: *Cyprinus Carpio L., zinc oxide nanoparticles, iron oxide nanoparticles.*

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Introduction

Aquatic organisms, especially fish, are considered essential nutrients for the growth and development of the human body. Fish is a major and important source of protein and essential amino acids that the human body needs (2). Zinc and iron are vital micronutrients necessary to maintain the normal body functions of all living organisms. They contribute to protein synthesis and act as an energy regulator. In addition, they help in fat metabolism (1). Fish is a major and important source of protein and essential amino acids that the human body needs (2). Fish feed is an important basic element in fish farming systems in particular and other aquatic organisms in general. Therefore, the feeds used in aquaculture must be supported to achieve growth, immunity, and health for these organisms; and to achieve the best weight gains in aquaculture systems. The quality of feed components is also considered one of the most important problems facing fish farmers, which in turn is reflected in growth performance and production costs (3). Balanced nutrition is the main factor for increasing production, which in turn requires providing the ideal needs of these nutritional components. However, the raw components, whether from plant or animal sources, in the basic diet do not provide sufficient quantities of minerals. Therefore, it is necessary to combine and add mineral elements at ideal concentrations and according to the needs of each type of animal with feed components, which vary according to the type of relationship, age, and size of the fish, as well as the method of farming the type to be raised (4). Zinc and iron are vital micronutrients necessary to maintain the normal body functions of all living organisms. They contribute to protein synthesis and act as an energy regulator. In addition, they help in fat metabolism(1). The incorporation of nanoparticles such as zinc oxide nanoparticles (ZnO-NPs) and selenium oxide nanoparticles (Se-NPs) in fish feed positively affects the fish farming process and improves their health and well-being. Nanomaterials have strong antioxidant properties play an effective role in biological functions such as immunity, fertility, metabolism, and contribute to stimulating many enzymes and wound healing. They also play an important role in growth performance and weight gain, and in alleviating oxidative stress in aquatic animals and fish (5). Nanoparticles were found to increase growth parameters and improve genes associated with fish growth, as these materials regulate gene expression and growth hormone (GH) (6). This study aimed to evaluate the effect of adding different levels of Fe_2O_3 and ZnO nanoparticles to the diet of common carp fingerlings on their growth parameters, feed efficiency, and physical properties.

Materials and Methods

Common carp fingerlings were purchased from a fish farm in Babylon Governorate/Al-Mahawil District/Al-Sabaghiyah Sub-district. A total of 200 fish were used in the experiment, with 182 individuals selected for the study, ranging in weight from 5 to 10 grams. The rest were kept as stock to compensate for the deaths that may occur before the start of the experiment and during the acclimatization period. Before starting the experiment, the fish were fed the control feed (or standard feed) for 10 days to standardize the nutritional status of the fish and ensure that the fish were subject to the same nutritional conditions to eliminate the effect of the previous feeds and produce the correct, clear and more accurate results. The experiment started from 14/7/2024 to 23/9/2024, where each treatment was fed separately, i.e. with a different feed from the other in terms of feed additives, nanomaterials, and their concentrations, as the first treatment was considered a control treatment, while the second was an addition to the control feed of a nanomaterial (iron oxide nanoparticles at a concentration of (30 mg/kg), while the third treatment (control feed + 30 mg/kg of zinc oxide nanoparticles) and the fourth treatment (control feed + 40 mg/kg of iron oxide nanoparticles) and the fifth treatment (control feed + 40 mg/kg of nano zinc oxide) and the sixth treatment (control feed + 30 mg/kg of nano iron oxide + 30 mg/kg of nano zinc oxide) and the seventh treatment (control feed + 40 mg/kg of nano iron oxide + 40 mg/kg From nano zinc oxide). The fish were given 0.03 of their weight daily and once a day and the weight of the fish was calculated every 14 days. The experiment continued for 70 days and then the amount of feed consumed, the total weight gain, and the conversion factor for each replicate were calculated.

Experimental materials

The nanomaterials (ZnO-NPs) and (Fe_2O_3-NPs) were purchased from Al-Bashir Scientific Office for Laboratory Supplies and Scientific Consultations located in Baghdad, Bab Al-Muadham. Note that the size of the nanomaterial (nano zinc oxide) ranges between (40 ± 5 nm) and the size of (nano iron oxide) ranges between (30 ± 10 nm). The feed consisted of the following materials (fish meal, soybean meal, yellow corn, flour, bran, rice flour, premix, and cooking oil). These materials were ground and crushed well using a Chinese electric grinder. Then the materials were mixed according to the proportion shown in Table (1). The materials were mixed manually and stirred more than once to ensure their homogeneity, then water (included nanoparticles) was added to them gradually to obtain a dough with a homogeneous and cohesive texture. The dough was processed into food pellets using a grinding machine with a 2 mm diameter and left for 4 days, stirring until it. According to the treatments, the young carp were fed with manufactured feed at a rate of 3% of the total weight of the fish.

Statistical Analysis

In the statistical analysis, the Complete Randomized Design (CRD) was used to study most of the productive traits of common carp fish cultured in breeding ponds by using the Statistical Package for Social Sciences (SPSS) version 26. And extracting the significant differences between the averages of the studied traits using Duncan's Multiple Range Test (1955) at significant levels (0.05) (7).

Table (1): The composition of the experimental feed provided to the fish during the study.

No.	Components	Mixing rate	Quantity/g
1.	Protein (Fish meal)	15%	150
2.	Proteins (Soybean meal)	30%	300
3.	Carbohydrates (Yellow corn)	10%	100
4.	Carbohydrates (Wheat flour)	20%	200
5.	Bran	10%	100
6.	Milling by-products	13%	130
7.	Premix	1.5%	15
8.	Fat	0.5%	5
9.	Total	100%	1000 g

Result and Discussion

Growth parameters

Initial and final weight gain rates in Cyprinus Carpio L.

According to the statistical analysis, as shown in Table (2), there were no significant differences for the initial weight of common carp fingerlings across all treatments. However, significant differences ($P \leq 0.01$) were observed for the final weight, with a notable superiority in the second treatment (T2), which received nano iron oxide at 30 mg/kg, outperforming all other treatments, including the control group. The fifth treatment (T5), which contained nano zinc oxide at 40 mg/kg, followed closely. Treatment T6 also showed significant improvement, while treatments T1, T4, T3, and T7 exhibited similar final weights. This study agreed with many previous studies, including (8). Stated environmental factors and the type of nutrition greatly affect metabolic rates and metabolism and also play an important role in the growth and overall weight gain (9).

Rates of daily and total weight gain

For the rate of daily and total weight gain, the results showed A highly significant superiority ($P \leq 0.01$) for the second treatment (T2, 30 mg/kg of nano iron oxide) overall treatments, followed by the fifth treatment T5 (40 mg/kg of nano zinc oxide) and then the fourth treatment T4, which showed significant similarity with the sixth and third treatments T6 & T3. As for the seventh treatment, it recorded a decrease in total and daily weight gain, and the lowest weight gain was recorded by the control treatment T1 by (81 g). The current study agreed with many previous studies and research, including the study of researchers (10), which indicated that the diet supplemented with nanoparticles Fe_2O_3 (40) mg/kg has a significant positive effect and an increase in the activities of digestive enzymes. This increase in digestion may lead to the consumption of large amounts of feed, which leads to increased feed efficiency, increased body weight, and ideal growth and increase. Another study by (11) showed that nano-iron oxide has a significant positive effect on metabolic rates, synthesis of essential amino acids, and feed conversion ratio (FCR).

Table (2): Effect of adding different levels of Fe_2O_3-NPs and ZnO-NPs Supplementation on Weight Gain in Common Carp (initial and final mass weight rates and daily and total gain).

Treatments	weight average ± standard error			
	Initial weight (g)	Final weight (g)	Total weight gain (g)	Daily weight gain (g)
T1	103.50 ± 0.50	184.50 d ± 1.50	81.0 d ± 1.0	1.15 d ± 0.01
T2	104.50 ± 1.50	217.50 a ± 0.50	113.0 a ± 2.00	1.61 a ± 0.02
T3	106.5 ± 0.500	196.0 c ± 3.0	89.5 c ± 2.50	1.27 c ± 0.03
T4	105.0 ± 1.0	196.5 c ± 1.5	91.5 c ± 2.5	1.30 c ± 0.03
T5	104.0 ± 1.0	205.5 b ± 0.5	101.50 b ± 0.5	1.45 b ± 0.007
T6	106.5 ± 0.50	197.0 c ± 2.0	90.5 c ± 2.50	1.29 c ± 0.03
T7	106.5 ± 1.5	192.5 c ± 2.5	86.5 dc ± 4.0	1.22 dc ± 0.05
significantly	N.S.	**	**	**

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.

Specific growth rates (%/day) and relative growth rates (%)

Regarding to the specific growth rates (%day) and relative growth rates(%), The results in Table (3) revealed that the second treatment (T2), with 30 mg/kg of Fe_2O_3-NPs, and the fifth treatment (T5), with 40 mg/kg of nano zinc oxide, showed significant superiority ($P \leq 0.01$). These two treatments exhibited a clear similarity in their specific growth rates. The third treatment (T3) also demonstrated a significant similarity to treatments T6, T1, and T7. In contrast, the fourth treatment (T4) showed a noticeable decreasing in terms of growth performance. The results also indicated a highly significant superiority of the second treatment T2 in the relative growth coefficient over all other different treatments, followed by the fifth treatment T5, and these two treatments are followed by the fourth treatment T4, as the results showed a significant similarity with the other different experimental treatments, the sixth, third, seventh and first treatments (T6, T3, T7 & T1) within the relative growth rate.

Table (3): The effect of adding Fe_2O_3-NPs and ZnO-NPs to common carp feed on growth rates (qualitative and relative) in different treatments. (mean ± standard error).

Treatments	weight average ± standard error	
	Specific growth rate (%/day)	Relative growth rate%
T1	2.85 b ± 0.0	78.25 c ± 0.58
T2	4.28 a ± 0.0	108.1 a ± 3.4
T3	2.85 b ± 0.0	84.02 c ± 1.95
T4	2.8 c ± 0.01	87.17 c ± 3.2
T5	4.28 a ± 0.0	97.6 b ± 1.41
T6	2.85 b ± 0.0	84.9 c ± 2.74
T7	2.85 b ± 0.0	80.82 c ± 4.8
Significantly	**	**

The current study was observed to be consistent with many previous studies and research. Researchers (12) indicated in their study on nano iron forms versus bulk iron as feed additives to fish feed and their effect on growth parameters and blood parameters, and morphological and histological analyses of the intestines of Nile tilapia Oreochromis niloticus, where the results of this recent study were an increase in final body weight FBW and an increase in relative weight rate and specific growth rate SGR, as well as the best performance of the FSH hormone was observed in the group of tilapia fish that were fed a diet containing nano iron oxide materials at a concentration of 40.

Food conversion rate

Table (4) showed a clear significant superiority of the second treatment T2 over all the experimental treatments with a feed conversion factor of 2.5 grams, followed by the fifth treatment T5, which showed a significant difference between it and the other treatments, as there were no significant differences between the fourth treatment and the third treatments T3 and the sixth T6, and the first treatment had the least superiority, followed by the seventh.

Food conversion efficiency (%)

While for food conversion efficiency, the results indicated a highly significant superiority for the second treatment T2, followed by the fifth T5 treatment. Moreover, there was a clear significant difference between them, and there was no significant difference between the fourth treatment T4, and the third treatment T3. The significance was also similar between the sixth and the seventh treatments, and the least superior was the first treatment T1.

Table (4): Effect of Fe_2O_3-NPs and ZnO-NPs on growth, feed efficiency, and protein utilization in *Cyprinus carpio L.*

Treatments	(mean ± standard error)				
	Amount of feed consumed (g)	Food conversion rate	Food conversion efficiency (%)	Protein intake (g/fish)	Protein efficiency ratio (%)
T1	266.2 b ± 1.75	3.29 a ± 0.02	30.42 d ± 0.17	79.73 c ± 0.47	43.9 b ± 0.18
T2	283.2 a ± 4.62	2.50 d ± 0.08	39.92 a ± 1.35	88.80 a ± 1.60	51.95 a 0.80
T3	269.36 b ± 6.16	3.01b ± 0.02	33.22 c ± 0.16	82.32bc ± 1.81	45.65 ab ± 0.57
T4	272.79 ab ± 1.47	2.97 b ± 0.06	33.53 c ± 0.73	82.04 bc ± 0.89	46.35 ab ± 3.64
T5	275.94 ab ± 1.68	2.72 c ± 0.03	36.78 b ± 0.40	84.33 b ± 1.48	49.39 ab ± 0.36
T6	275.24 ab ± 2.24	3.04 b ± 0.06	32.87 cd ± 0.64	85.21 ab ± 0.61	45.93 ab ± 0.80
T7	272.65 ab ± 4.69	3.17 ab ± 0.09	31.52 cd ± 0.92	84.63 ab ± 1.29	44.71 b ± 2.78
significantly	N.S.	**	**	N.S.	N.S.

Regarding to above, this study showed that the nanomaterial has a positive effect on weight increases specific growth rates (SGR) and relative growth rates (RGR). This is consistent with the results of the previous study conducted by (13). This confirmed that the integration of nanomaterials into fish feed provides many benefits, including improving the absorption of nutrients, enhancing the specific growth rates (SGR) and relative growth rates (RGR), improving feed conversion rates, and improving the general health of fish.

From this, it can be said that determining the concentration of nanomaterials is important and necessary in fish farming. Here, the important role of the size of the nanomaterial is evident. The smaller the size of the nanomaterial, the higher its effectiveness and the higher its toxic effect. This is consistent with the study conducted by the researcher (14). (15) stated that growth criteria are basic scientific criteria for evaluating feed quality and protein levels.

Conclusion

Through the current study, the following was concluded:

Adding nano-materials, nano iron oxide, and nano zinc oxide, to common carp fish feed at specified concentrations has an effective and positive role and improves growth parameters, The present study proved that nano zinc oxide at a concentration of 30 mg/kg and a size of (20-40 nm) nanometers and nano zinc oxide at a concentration of 40 mg/kg and a size of (35-45 nm) nanometers have an effective effect on the total weight gain and daily gain and improve the feed conversion efficiency and the food conversion rate consumed by fish. this result suggests conducting histological examinations of fish fed nanomaterials and determining their effect on the fish.

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