



The effects of Selenium Nanoparticles and Stocking Density on Growth Performance of Common Carp (*Cyprinus Carpio*)

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ABSTRACT

There is a pressing need to design nutritionally balanced diets tailored to the specific requirements of fish cultured under varying aquaculture conditions in Egypt. Selenium (Se) is an essential trace element for animals, including fish. Nano-Se is more stable and soluble. The present study was conducted to investigate the effects of selenium nanoparticle supplementation and stocking density on growth performance traits, feed utilization and chemical body composition, of Common carp (*Cyprinus carpio*). Fish were randomly allocated according to a 2×5 factorial experimental design, consisting of two stocking densities (15 and 30 fish per aquarium) each combined with five dietary levels of Nano-selenium supplementation (0, 1, 3, 5, and 7 mg/g), resulting in 10 treatments with three replicates for each. Nano-selenium supplementation significantly improved ($P < 0.01$) fish growth performance, including final body weight, weight gain, and specific growth rate (SGR). The maximum fish growth, highest feed intake, and the lowest feed conversion ratio (FCR) were obtained when fish were fed a diet containing 3, 5 mg Nano-Se/Kg. Moreover, Nano-Se supplementation reduced the stress of stocking densities. Nano-selenium levels and stocking density significantly affected fish body composition and survival rate. Optimal improvements in protein content and survival rate were observed at 3–5 mg/kg Nano-Se, particularly under low stocking density. It could be concluded that Nano-Se supplementation, 3, 5 mg Nano-Se/Kg, enhanced the growth, feed utilization, and body composition of Common Carp fish.

Keywords: Common Carp (*Cyprinus carpio*), selenium nanoparticles, stocking densities, growth performance, feed utilization, body composition, Blood Component.

INTRODUCTION

Aquaculture is the fastest-growing food production sector, contributing to rural livelihoods while relieving pressures on wild fishery resources (FAO, 2016). In 2020, aquaculture production reached a second record high of approximately 123.3 MM, despite the impact of the COVID-19 pandemic (FAO, 2022). Approximately, 58.5 million people were documented as being directly engaged in the fisheries and aquaculture sector in 2020 (Murekezi *et al.*, 2020). Meanwhile, it has also been noted that approximately 600 million people have their livelihoods dependent on this sector (Murekezi *et al.*, 2020). These achievements are largely attributed to the intensification and expansion of farmed aquatic species. However, sustaining this growth necessitates continuous advancements in aqua-feeds production, including cost reduction and innovative technologies to address the growing needs for superior-quality aqua-feeds.

Over the past two decades, aquaculture has become a more significant contributor to the world's fish production. Production peaked in 2014 at 73.8 million tons, or approximately 44% of the world's total fish production. Aquaculture accounted for almost half of the historic high of 179 million tons of production from catch fisheries and aquaculture combined in 2018. Additionally, aquaculture has been the fastest-growing animal food industry over the last decade, accounting for 52% of fish produced for human consumption. A larger share of this development in aquaculture output can be attributed to production in Asia and Africa. Even in countries where the economy has been challenging and environmental issues persist, aquaculture helps support livelihoods, promote economic growth, reduce poverty, and enhance human food security, while also generating income. The aquaculture industry must continue to expand in order to meet the growing demand for fish. However, without a major improvement in planning and administration, development would not be sustainable. To address environmental, social, economic, health, and welfare issues related to animals, planning and management at local, national, and international levels are required (Salin & Arome Ataguba, 2018; FAO, 2020).

More than 80% of fish raised for food are carps, which include the common carp, silver carp, and grass carp, as well as minor and exotic carps, such as the rohu, catla, and mrigal. Over the past decade, carp production has increased by nearly 2 million tons, largely due to the adoption of enhanced aquaculture

practices. Fish are the dominant product in this sector, with the common and silver carp being the two most significant species by volume. For example, in 2018, global production of the silver carp reached 4,822,794 tons. The leading producers of these species include China, India, Bangladesh, and Iran (Molnár *et al.*, 2018; FAO, 2022).

In recent years, selenium nanoparticles (Se-NPs) have emerged as a novel form of Se supplementation in aquafeeds, offering several advantages over traditional organic and inorganic Se compounds. One of the main benefits of Se-NPs is their higher bioavailability, which ensures more efficient absorption and utilization by farmed aquatic species. This enhanced bioavailability translates to lower required concentrations and reduced toxicity, minimizing the risk of Se accumulation and toxicity (Kumar *et al.*, 2017).

This work aimed to study the effect of selenium nanoparticle supplementation and stocking density on growth performance and feed utilization of Common Carp *Cyprinus carpio*.

Materials & Methods

2.1 Fish and experimental design

This study was designed to investigate the effect of adding Nano-selenium and stocking density on the growth of carp fish, where 4 different concentrations selenium Nano particles (prepared from sodium selenite by wet chemical method and characterized by SEM and HRTEM) (Preparation was done at Naqaa Foundation for Scientific Research, Technology and Development, Giza, Egypt) were used in addition to the control (0,1,3,5,7 mg/kg) and the fish were randomly distributed into two groups with a stocking density (15,30 fish/Aquaria). For each treatment, three replicates were used.

This study was conducted using 30 glass Aquaria, with an area of 80 × 30 × 40 cm. Before starting the study, the Aquaria were washed and disinfected thoroughly and filled with well water, leaving a 10cm area at the top of the Aquaria. The water is replaced by 50% daily. All ponds were provided with pneumatic hoses, and all Aquaria were covered with nets to reduce the escape of fish. Fingerlings were used in this study, with an average initial weight of 2 g per fish, obtained from the Fish Hatchery in Abbassa, Sharkya Governorate, Egypt. The fish were homogeneous in body weight and appeared to be healthy; any unhealthy fish were removed. Fish were acclimated to experimental conditions for 2 weeks before the experiment, where they were fed a basic diet.

2.2 Experimental diet:

The diets were formulated from practical ingredients. The experimental diets were prepared by individually weighing each component and thoroughly mixing the minerals, vitamins, and additives to homogenize them. The mixture was then divided into five portions until it was supplemented with nano selenium at different concentrations. After complete characterization of the nanoparticles, the suggested levels used were 0, 1, 3, 5, and 7 mg/kg diet. Therefore, it was necessary to suggest a more applicable and accurate method for mixing nano selenium particles into the diet. Distilled water was used as a vehicle to mix the selected concentration of nanoparticles in the experimental diet. It was achieved by mixing 1 gram of selenium nanoparticles with half a liter of distilled water, then thoroughly mixing to ensure a homogeneous distribution of nanoparticles throughout the mixture. So, we achieved the actual concentration of each mL of this mixture contains 2 mg of Nano selenium

Table(1): Ingredient and Chemical composition of the experimental diets

Ingredient (kg)	100kg
Fish meal	11
Soybean meal	41
Wheat bran	17
corn starch	17
Rice bran	7
Vitamin premix (1)	1.5
Minerals premix (2)	1.5
corn oil	2
Starch	2
Chemical composition (%DMB)	
Moisture	9.5
Crude protein	33
Crude lipid	18.2
Ash	10
Crude fiber	9.3
NFE ³	29.5
Gross energy	479.69

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1. Each 3 kg of vitamin mixture contained: Vit. A 120000IU, D 300000IU, B1 500 mg, B2 200mg, B6 600mg, B12 3mg, E 700mg, C 450mg, K 500mg, Pantothenic acid 670mg, Folic acid 300mg, Biotin 6 mg and Choline chlorides 10000mg, 2-Each three Kg of mineral mixture contained: Zinc sulfide 1800mg, Iron sulfide 10000mg, Copper sulfide 3000mg, Magnesium sulfide 3000mg, Cobalt sulfide 300mg 3-Nitrogen free extract =100-(% protein + % fat +% fiber +% Ash) 4-Gross energy based on protein (5.65 kcal/g), fat (9.45 kcal/g) and Carbohydrate 4.11 kcal/g. According To (NRC, 1993)

The biweekly bulk weight in each pond was used to readjust the biweekly ration of the fish. Throughout the study period, the physicochemical parameters of the water were measured, along with the growth performance of the fish. Randomized fish samples were collected from the ponds at the end of the study to determine the proximate composition (whole-fish body) according to the standard methods of AOAC (1995). Statistical analysis was performed using the 2 x 5 factorial design. Values are given as Mean + SE. Means were tested for significant differences at P-values < 0.05 (Duncan, 1955). All statistical analyses were performed using the SPSS program version 20 (SPSS, Richmond, USA), as described by Dytham (1999).

Result and discussion

Results of growth performance showed final body weight (g), weight gain (g), average daily gain (g/fish/day), and specific growth rate (%/day). No significant difference was found between experimental treatments for initial live body weight, indicating that the groups at the beginning of the experiment were homogeneous (Table 2). Fish were fed diets supplemented with 0, 1, 3, 5, and 7 mg Nano-Se/kg for 12 weeks. The growth performance of common carp fed diets supplemented with varying levels of dietary Nano-Se is presented. Fish that were fed a diet supplemented with 3 and 5 mg Se/kg displayed improved ($P \leq 0.001$) growth performance, including final weight, weight gain (WG), Average daily gain (ADG), and Relative gain (RG). In contrast, the specific growth rate (SGR) was significantly affected ($P \leq 0.05$) in fish fed a diet supplemented with 5 and 7 mg Se/kg. No significant differences were observed between low and high stocking densities in growth performance. Selenium is an essential element for normal growth and development in fish (Hamilton, 2004). The optimum Se requirement for common carp determined in this study (3 and 5 mg nano-Se/kg) is higher to that reported for gibel carp (1.18 mg Se/kg; Han *et al.*, 2011), and higher than that determined for loach (0.48-0.50 mg Se/kg; Hao *et al.*, 2014 and grouper (0.77 mg Se/kg; Lin and Shiau, 2005). Thus, the difference in Se requirement

could also be related to the species and age of fish, the chemical forms of Se, and dietary factors (**Hao *et al.*, 2014**).

The final body weight at the end of the experiment was not significantly affected by the difference in stocking density between low and high, nor by the treatments or Nano-Se concentrations (Table 2). Additionally, the addition of nano-selenium mitigated the increase in density. Additionally, it reduces the stress resulting from high stocking densities.

Due to the combined and interactive effects of stocking density and treatments, the average final body weight was significantly affected ($P \leq 0.001$). The maximum values were observed in T3 and T4 in both low and high density (12.23, 12.47, 12.33, and 12.43g, respectively) as shown in Table 2. **Saffari *et al.* (2017)** observed that the initial weight between the experimental groups of fish fed diets with 0.7 mg Nano-Se/kg had the highest final weight of common carp. Our results are the same (**Neamat-Allah *et al.*, 2019**), which showed that the effect of varied forms of Se on the growth performance of Nile tilapia final body weight was higher in the Nano-Se group than in the CT.

Previous study indicated that high levels of survival may be due to low feed competition and density (**Narejo *et al.*, 2005**). The present study stated that survival was not affected by any stocking density. This finding is similar to some research that suggests the survival of fish is independent of stocking density (**Keshavanath *et al.*, 2015**). Additionally, the survival of fish in practical culture may also depend on the specific species. For example, the survival of the catfish *Rita rita* at different densities (10, 20, and 30 fish per cistern) resulted in the highest survival rate at 20 fish per cistern. In contrast, a prior study revealed that the survival rate in aquatic animals was negatively correlated with stocking, which could be due to high competition and limited space for the fish (**Daudpota *et al.*, 2014**).

The crude protein (CP) was not affected significantly ($P \leq 0.05$) between low and high stocking density, and was affected significantly ($P \leq 0.001$) among the Nano-selenium treatments where the highest value was recorded at T5 then T2, (Table 3) concerning the combined effects between stocking density and treatments, a significant ($P \leq 0.001$) difference was observed. The highest value at T5 was documented at both high and low densities (61.13 and 61.01, respectively). Crude lipid was significantly affected ($P \leq 0.001$), increasing with increasing density. There was a slight, but significant ($P \leq 0.05$) difference between treatments or Nano selenium concentrations, with the highest value for T1. There was a significant effect ($P \leq 0.001$) due to the interaction between stocking density and treatments.

Maximum values were observed at T1 and T3 at the higher density (33.63, 33.63) (Table 3).

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Table (2): Growth performance of common carp as affected by stocking density, Nano selenium concentration, and their interaction.

Items	Initial Weight	Final Wight	Wight gain	ADG	RG	SGR
Effect of stocking density						
Low	2.12±0.0 1	11.37±0.2 7	9.25±0.27	0.11±0.00	435.88±15.8 8	2.35±0.1 2
High	2.07±0.0 1	11.27±0.2 7	9.20±0.27	0.11±0.00	449.18±13.1 9	2.22±0.0 8
Significance	NS	NS	NS	NS	NS	NS
Concentration of Nano selenium						
T1(C)	2.08±0.0 3	10.10 ^c ±0.2 1	8.02 ^c ±0.2 3	0.10 ^c ±0.0 0	385.65 ^c ±14. 94	1.88 ^b ±0. 05
T2	2.08±0.0 2	10.63 ^{bc} ±0. 28	8.55 ^{bc} ±0. 28	0.10 ^{bc} ±0. 00	410.32 ^{bc} ±12 .26	2.28 ^{ab} ±0. 24
T3	2.10±0.0 3	12.28 ^a ±0.1 4	10.18 ^a ±0. 15	0.12 ^a ±0.0 0	485.51 ^a ±11. 38	2.27 ^{ab} ±0. 13
T4	2.10±0.0 3	12.45 ^a ±0.1 1	10.35 ^a ±0. 12	0.12 ^a ±0.0 0	493.38 ^a ±9.8 4	2.35 ^a ±0. 04
T5	2.10±0.0 0	11.13 ^b ±0.1 5	9.03 ^b ±0.1 5	0.11 ^b ±0.0 0	430.16 ^c ±6.9 2	2.65 ^a ±0. 10
Significance	NS	***	***	***	***	*
Interaction between Stocking density and Nano selenium concentration						
Low Stocking density						
T1(c)	2.13 ^a ±0. 03	10.00 ^d ±0.1 5	7.78 ^d ±0.1 3	0.09 ^d ±0.0 0	368.83 ^d ±6.0 8	1.86 ^b ±0. 07
T2	2.10 ^{ab} ±0. 00	10.83 ^{bcd} ±0. 52	8.73 ^{bcd} ±0. 52	0.10 ^{bcd} ±0. 01	415.87 ^{cd} ±24 .95	2.36 ^{ab} ±0. 48
T3	2.13 ^a ±0. 03	12.23 ^a ±0.1 8	10.10 ^a ±0. 21	0.12 ^a ±0.0 0	473.95 ^{ab} ±16 .75	2.36 ^{ab} ±0. 04
T4	2.13 ^a ±0. 03	12.47 ^a ±0.1 5	10.33 ^a ±0. 18	0.12 ^a ±0.0 0	484.85 ^{ab} ±15 .40	2.34 ^{ab} ±0. 07
T5	2.10 ^{ab} ±0. 00	11.33 ^b ±0.2 4	9.23 ^b ±0.2 4	0.11 ^b ±0.0 0	439.68 ^{bc} ±11 .45	2.82 ^a ±0. 14
High Stocking density						
T1(c)	2.03 ^b ±0. 03	10.20 ^{cd} ±0. 44	8.17 ^{cd} ±0. 46	0.10 ^{cd} ±0. 01	402.46 ^{cd} ±28 .21	1.90 ^b ±0. 10
T2	2.07 ^{ab} ±0. 03	10.43 ^{bcd} ±0. 30	8.37 ^{bcd} ±0. 27	0.10 ^{bcd} ±0. 00	404.76 ^{cd} ±9. 91	2.20 ^{ab} ±0. 23
T3	2.07 ^{ab} ±0. 03	12.33 ^a ±0.2 6	10.27 ^a ±0. 26	0.12 ^a ±0.0 0	497.06 ^a ±15. 28	2.19 ^{ab} ±0. 27
T4	2.07 ^{ab} ±0. 03	12.43 ^a ±0.2 0	10.37 ^a ±0. 20	0.12 ^a ±0.0 0	501.90 ^a ±13. 21	2.35 ^{ab} ±0. 06
T5	2.10 ^{ab} ±0. 00	10.93 ^{bc} ±0. 09	8.83 ^{bc} ±0. 09	0.11 ^{bc} ±0. 00	420.64 ^{cd} ±4. 20	2.28 ^{ab} ±0. 05

Significance	*	***	***	***	***	*
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Mean values of the same column within each part of the table with different superscripts are significantly different, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, and NS = Non-significant.

Table (3): Feed utilization of common carp as affected by stocking density, Nano selenium concentration, and their interaction.

Items	Feed utilization		
	FI	FCR	FER
Effect of stocking density			
Low	18.18±0.23	1.98±0.05	50.85±1.19
High	18.13±0.31	1.98±0.04	50.64±0.92
Significance	NS	NS	NS
Concentration of Nano selenium			
T1(C)	17.56 ^b ±0.36	2.19 ^a ±0.04	45.64 ^c ±0.73
T2	17.40 ^b ±0.49	2.04 ^b ±0.07	49.28 ^b ±1.88
T3	18.93 ^a ±0.22	1.86 ^c ±0.04	53.84 ^a ±1.07
T4	19.25 ^a ±0.14	1.86 ^c ±0.02	53.77 ^a ±0.46
T5	17.65 ^b ±0.11	1.96 ^{bc} ±0.04	51.20 ^{ab} ±0.99
Significance	**	***	***
Interaction between stocking density and nano selenium concentration			
Low Stocking density			
T1(c)	17.58 ^{cb} ±0.49	2.23 ^a ±0.04	44.78 ^b ±0.77
T2	17.66 ^{cb} ±0.47	2.04 ^{abc} ±0.15	49.62 ^{ab} ±3.99
T3	18.72 ^{ab} ±0.17	1.85 ^c ±0.02	53.94 ^a ±0.64
T4	19.30 ^a ±0.20	1.87 ^c ±0.03	53.54 ^a ±0.77
T5	17.64 ^{cb} ±0.24	1.91 ^c ±0.06	52.38 ^a ±1.79
High stocking density			
T1(c)	17.54 ^{cb} ±0.63	2.15 ^{ab} ±0.05	46.50 ^b ±1.16
T2	17.14 ^c ±0.95	2.05 ^{abc} ±0.05	48.94 ^{ab} ±1.27
T3	19.14 ^a ±0.40	1.87 ^c ±0.08	53.73 ^a ±2.29
T4	19.20 ^a ±0.24	1.85 ^c ±0.02	53.99 ^a ±0.64
T5	17.66 ^{cb} ±0.05	1.99 ^{bc} ±0.02	50.02 ^{ab} ±0.50
Significance	*	**	**

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Ash was significantly affected ($P \leq 0.001$) by stocking density, as it increased with lower density. Furthermore, there were significant differences ($P \leq 0.001$) between the treatments, with the maximum values observed at T5. As a consequence of the combined effects of stocking density and treatments, ash was affected significantly ($P \leq 0.001$), and the maximum values of T5 were at both low and high densities (14.07, 14.50). Regarding moisture, the results obtained at the end of the experiment indicated that stocking density significantly affected ($P \leq 0.001$) the outcome, with higher density resulting in increased moisture. In carp culture, high stocking densities create very high moisture levels because more fish in a given area excrete more waste, which is rich in moisture, and also due to the potential for increased feeding and overall biological activity that consumes oxygen and produces carbon dioxide and other dissolved organic matter, which can also be interpreted as very high moisture in Nano selenium treatments where the highest value is documented in T2 and T3, with Concerning the combined effects between density and treatments. They were significantly affected ($P \leq 0.001$), with the highest value observed at T5 at high density (80.43).

Proximate composition of fish was affected by the dietary treatment after 12 weeks of culture, indicating that the carp muscle proximate Composition is sensitive to dietary Se treatments. This observation is in not agreement with the results obtained by (Zhou *et al.*, 2009) for crucian carp and (Le and Fotedar. 2014) for juvenile yellowtail kingfish whose reported that the carp muscle proximate Composition is not sensitive to dietary Se treatments A significant increase in the muscle fiber number, reflected by a higher muscle fiber density, is indicative of favorable growth conditions Rowlerson, 2001.

Table (4): Body composition (%) and Survival rate of common carp as affected by stocking density, Nano selenium concentration, and their interaction for all treatments as %DMB.

Items	Body composition				
	Crude protein	Crude Lipid	Ash	Moist	SR
Effect of stocking density					
Low	58.57±0.80	26.05±0.57	14.20±0.24	76.93±0.51	82.65±1.57
High	57.13±1.10	30.07±0.90	11.23±0.24	79.39±0.25	83.21±1.44
Significance	NS	***	***	***	NS
Concentration of Nano selenium					
T1(C)	56.43 ^b ±0.29	31.59 ^a ±0.94	11.00 ^b ±1.06	76.90 ^b ±0.40	79.98 ^{bc} ±1.93
T2	59.08 ^a ±1.01	27.72 ^{ab} ±1.4 ₃	11.73 ^b ±0.39	79.42 ^a ±0.07	83.87 ^{ab} ±1.33
T3	55.13 ^b ±0.29	29.42 ^{ab} ±2.4 ₄	12.72 ^b ±1.16	79.30 ^a ±0.25	88.32 ^a ±2.06
T4	57.00 ^b ±1.76	28.88 ^{ab} ±1.8 ₆	13.20 ^b ±0.37	77.83 ^{ab} ±0.76	85.27 ^{ab} ±2.21
T5	59.75 ^a ±0.18	25.00 ^b ±0.18	14.23 ^a ±0.10	77.37 ^{ab} ±1.38	77.20 ^b ±1.35
Significance	***	*	**	*	**
Interaction between Stocking density and Nano selenium concentration					
Low stocking density					
T1(c)	56.33 ^d ±0.03	29.33 ^c ±0.09	13.07 ^b ±0.18	76.03 ^c ±0.13	80.00 ^{bc} ±3.87
T2	58.07 ^c ±0.23	27.73 ^c ±0.12	12.90 ^c ±0.12	79.40 ^b ±0.10	84.40 ^{abc} ±2.20
T3	55.88 ^{ef} ±0.22	27.80 ^c ±0.15	13.40 ^b ±0.12	78.77 ^c ±0.03	86.63 ^{ab} ±3.84
T4	60.67 ^b ±0.03	25.63 ^f ±0.15	13.20 ^b ±0.17	76.17 ^c ±0.23	84.43 ^{abc} ±4.43
T5	61.13 ^a ±0.24	24.27 ^g ±0.09	14.07 ^a ±0.09	74.30 ^f ±0.27	77.77 ^{bc} ±2.23
High stocking density					
T1(c)	56.17 ^d ±0.63	33.63 ^a ±0.12	8.43 ^h ±0.03	77.77 ^d ±0.17	79.97 ^{bc} ±1.93
T2	61.70 ^a ±0.29	28.50 ^d ±0.06	9.21 ^d ±0.07	79.43 ^b ±0.12	83.33 ^{abc} ±1.93
T3	55.70 ^c ±0.20	33.63 ^a ±0.09	9.33 ^d ±0.07	79.83 ^b ±0.17	90.00 ^a ±1.91
T4	53.73 ^f ±1.13	32.53 ^b ±0.03	12.20 ^c ±0.18	79.50 ^b ±0.20	86.10 ^{ab} ±2.01
T5	61.01 ^a ±0.32	24.23 ^g ±0.15	14.50 ^a ±0.18	80.43 ^a ±0.13	76.63 ^c ±1.93
Significance	***	***	***	***	*

Mean values of the same column within each part of the table with different superscripts are significantly different, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, and NS = Non-significant.

Conclusions

Effects of different levels of Nano selenium on common carp were investigated. This research demonstrated that supplementation of Nano-Se in the diet at a rate of 3-5 mg/kg could enhance growth performance, feed utilization, and body composition in Common carp (*Cyprinus Carpio*) fish. Moreover, the addition of Nano-selenium reduced the effect of increasing the density. Furthermore, it reduces the stress resulting from stocking densities. The data of the present study recommend adding 3, 5 mg Nano-Se per kg diet to improve carp growth.

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