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Effects of chromium nanoparticles supplementation on body composition of *Cirrhinus mrigala* fingerlings fed sunflower meal based diet

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Abstract

Nanoscience is a fast growing field of research which aims to generate new materials having wide range of applications. This research was conducted to evaluate the effects of Cr nanoparticles supplemented sunflower meal based diet on body composition of *Cirrhinus mrigala* fingerlings. Nano Cr was added in sunflower meal based diet having graded (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg Kg^{-1}) levels. Fifteen fingerlings were stocked in each replicated V-shaped tank and fed at the rate of 5% of their live wet weight. Effects of each treatment on the carcass composition were calculated by standard methods and formulae. The best results in regard of body composition (CP; 59% and EE; 14%) were noted in fingerlings when fed on 2 and 1.5 mg Kg^{-1} levels of Cr-nano particles based diets, respectively. It was concluded from the results of current study that supplementation of Cr NPs (1.5-2 mg Kg^{-1}) in sunflower meal based diet improves the body composition of *C. mrigala* fingerlings.

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Introduction

The most important challenge to improve the aquaculture industry is the formulation of cost effective fish feed from high quality protein sources (Baruah *et al.,* 2004). Fishmeal was being used as a source of essential nutrients and growth factors in fish feed (Zhou *et al.,* 2004). Due to a number of reasons such as uneven supply, increasing demand and rising prices of the fish meal, it became necessary for researchers to search for the alternative protein sources for fish feed industry (Lech and Reigh, 2012). During research it was found that plant by-products are the best source of protein and energy to make economical and environment friendly fish feed (Gatlin *et al.,* 2007). There was a problem with the use of low cost plant proteins in fish feed and that was the presence of ingredients with larger sizes which decrease the digestibility of fish. While in digestion, nutrients having larger sizes have to convert into small particle size, so that they can pass easily through the intestinal mucosa. In feed industry nano-particles are being used in feed processing and dietary supplementations (Powell *et al.,* 2010). Nanotechnology tools are being used by aquaculture industry for rapid disease detection and targeted delivery of nutrients (Ashraf *et al.,* 2011).

Nanotechnology will play an important role in the future areas of research in animal nutrition (Bunglavan *et al.,* 2014). The ability of nanoparticles to enhance protein stability may impact numerous biological processes such as digestion, metabolism and nutrient uptake (Sharma *et al.,* 2007). Chromium (Cr) is an essential trace element for humans and animals. It is associated with the metabolism of carbohydrates, lipids, nucleic acids and proteins (Wang and Xu, 2004). Its deficiency results in growth retardation. The absorption and utilization of Cr may be dependent on its status in intestinal tract. The properties of nanoparticles may bring unique potential applications. There is minute work on the effects of Cr supplementation on pork quality and individual muscle weight (Wang and Xu, 2004). Cr-Nano dietary supplementation also increases serum insulin-like growth factor I and reduces serum insulin and cortisol levels. Moreover, Cr-Nano results in significant increments of immunoglobulin contents in plasma (Wang *et al.,* 2007).

It also results in higher carcass lean percentage and lower carcass fat percentage. In addition, supplemental Cr-Nano increases Cr concentration in liver, kidney and heart significantly. Supplemental Cr-Nano has beneficial effects on carcass characteristics (Wang and Xu, 2004).

In Pakistan, the production of sunflower is carried out to fulfill the demands of country for edible oil. This oil-seed crop is available at low cost as compared to other protein sources (Khan *et al.,* 2006). Sunflower meal contains endogenous proteolytic enzymes, which help in the digestion of phytate bound proteins for fish (Kocher and Choct, 2000). Its efficiency is better than wheat bran and maize gluten in gaining maximum body weight in *C. mrigala* and *L. rohita* (Inayat and Salim, 2005).

It contains about 45-48% crude protein on oil extraction (Mushtaq *et al.,* 2006). The major goal of the current research was to estimate the effects of Cr nano-particles on body composition of *C. mrigala* fingerlings as well as to help the world in producing environment friendly fish feed.

Materials and methods

The present research was carried out in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad (latitude 31.4166° North and the longitude 73.0707° East).

Fish and experimental conditions

C. mrigala fingerlings were purchased from Government Fish Seed Hatchery, Faisalabad and were acclimatized to Laboratory conditions for 15 days in V-shaped tanks. During this period the fingerlings were fed once daily to apparent satiation on the basal diet used in subsequent digestibility study (Allan and Rowland, 1992). To maintain the level of dissolved oxygen, air was supplied through capillary system.

Feed ingredients and experimental diets

The feed ingredients were purchased from a commercial feed mill and were analyzed for chemical composition by following standard methods prior to the formulation of the experimental diets (AOAC, 1995). The basal diet was prepared to supply adequate levels of required nutrients for normal fish growth (Divakaran *et al.,* 2002).

Formation of feed pellets

The feed ingredients were finely grinded to pass through 0.5 mm sieve size. All ingredients were mixed in a mixer for 5 minutes and fish oil was gradually added thereafter. Water was slowly added into the mixer, resulting in suitably textured dough that was processed by pelleting machine to make pellets (Lovell, 1989).

Preparation NP stock solution

Cr NPs were purchased from market (Sigma-Aldrich), the preparation and confirmation of stock solutions of NPs was carried out according to Federici *et al.* (2007) and Ramsden *et al.* (2009). Stock solution of 100% pure NPs dry powder was made by sonication method (for 6-8 hours) and from these stock solutions further dilutions were made to ensure our required levels of NPs (0, 0.5, 1, 1.5, 2, 2.5 and 3 $mgKg^{-1}$).

Addition of NPs to basal diets

The diluted NPs solutions were sonicated for further 15 minutes just before spraying to ensure even delivery of the material through the spray nozzle. One Kg of fish feed was kept in a commercial feed mixer (Kenwood Catering Professional food mixer XKM810) and was gradually sprayed with the appropriate dilution of NP solution. The NPs immediately coated the feed. The pellets were allowed to dry, after which the feed was transferred into airtight containers for storage. The control diet was prepared in exactly the same way, except that the NPs solution was replaced by an equal volume of ultrapure water. NPs concentrations in the diets were confirmed by following the nitric acid digestion method (Ramsden *et al.,* 2009).

Feeding Protocol

Fingerlings were fed at the rate of 5% of their live wet weight on their prescribed diets twice daily (morning and afternoon). Triplicate tanks were assigned for each experimental diet and in each replicate 15 fingerlings were stocked. After the feeding session of two hours the tanks were washed completely to remove the feed particles and refilled with water. The experiment was lasted up to 90 days.

Chemical analysis of fish whole body

At the end of 90 days trial, after taking blood, four fish from each tank were sacrificed randomly and dried at room temperature. Moisture contents of carcass were calculated after oven-drying of homogenised samples at 105°C for 12 hours. Micro kjeldahl apparatus (InKjel Mbehr Labor Technik GmbH D-40599 Dusseldorf) was used to determine the crude protein (CP) ($N \times 6.25$) whereas soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) was used to check the amount of crude fat by the help of petroleum Ether Extraction (EE) Method. Crude fiber contents were calculated as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH whereas ash was determined by ignition at 650°C for 12 hours in electric furnace (Naberthern B170) to constant weight. Total carbohydrates (N-free extract) were determined by difference, i.e., Total carbohydrates % =100- (EE % + CP % + Ash % + CF %). Oxygen Bomb Calorimeter was used to estimate the gross energy.

Statistical analysis

Finally data of body composition of fish was subjected to one-way analysis of variance (Steel *et al.,* 1996). The differences among treatments were compared by Tukey's Honesty Significant Difference Test and considered significant at $p<0.05$ (Snedecor and Cochran, 1991). The CoStat Computer Package (version 6.303, PMB 320, Monterey, CA, 93940 USA) was used for statistical analysis.

Results

Proximate composition (%) of *C. mrigala* carcass fed on Cr-nano supplemented Sunflower meal based diets is shown in Table 3. All the parameters are significantly ($p<0.05$) different from each other. Maximum values of CP (59%) and EE (14%) were observed in fish fed at 2 and 1.5 mg Kg^{-1} Cr-nano diets respectively followed (58 % and 14 % respectively) by fish that fed at 2.5 mg Kg^{-1} Cr-nano diet (T_6). These values are significantly ($p<0.05$) different from that of control group (CP: 54% and EE: 9%).

Table 1. Chemical composition (%) of feed ingredients.

Ingredients	Fish meal	Rice polish	Wheat flour	Sunflower meal
Dry matter (%)	91.67	94.06	92.4	93.80
Crude Protein (%)	49.03	11.87	9.73	40.81
Crude Fat (%)	6.93	12.69	2.24	3.69
Crude Fiber (%)	1.23	11.91	2.73	1.94
Ash(%)	23.15	11.32	1.99	9.96
Gross Energy (kcal/g)	2.49	3.41	3.06	3.64
Carbohydrates	19.66	52.21	82.21	43.6

The least CP (54%) and EE (9%) contents in fish body were observed when fed test diet $T_{7,}$ 3 mg Kg^{-1} Cr-nano. From results, it was found that improvement in protein and fat contents for *C. mrigala* fingerlings was started from 0.5 mg Kg^{-1} Cr-nano diet and reached its maximum in fingerlings fed 2 and 1.5 mg Kg^{-1} level of Cr-nano (respectively) supplemented Sunflower meal based diet. Further increase in Cr-nano supplementation could not enhance nutrient contents in fish body.

Minimum amount of carbohydrate (13%) and crude fiber (1%) contents were analyzed in fish fed at 2 and1.5 mg Kg^{-1} (respectively) Cr-nano level based diets as compared to other test diets and control diet (0 mg Kg^{-1} Cr-nano level in based diet).

Table 2. Ingredients composition (%) sunflower meal based test diets.

Ingredients	Test Diet-I	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI	Test Diet-VII
Nanoparticles (mg kg^{-1})	0	0.5	1	1.5	2	2.5	3
Sunflower meal	50	50	50	50	50	50	50
Fish meal	14.5	14.5	14.5	14.5	14.5	14.5	14.5
Wheat flour*	13	13	13	13	13	13	13
Rice polish	11	11	11	11	11	11	11
Fish oil	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Vitamin premix**	1	1	1	1	1	1	1
Minerals premix***	1	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1

* Nano-particles will be added on the cost of wheat flour

** Vitamin premix/kg= Vitamin D_3: 3,000,000 IU, Vitamin A: 15,000,000 IU, Vitamin E:30000 IU, Vitamin B_1: 3000 mg, Vitamin B_6: 4000 mg, Vitamin B_{12}: 40mg, Vitamin B_2: 7000 mg, Vitamin C: 15,000 mg, Vitamin K_3: 8000 mg, Folic acid: 1500 mg,Calcium pantothenate: 12,000mg, Nicotinic acid: 60,000 mg.

*** Mineral premix/kg= Mn(Manganese): 2000 mg, Ca (Calcium): 155 gm, Zn (Zinc): 3000 mg, Cu: (Copper), 600 mg, Co: (Cobalt), 40 mg, I (Iodine): 40 mg, P (Phosphorous): 135 gm, Fe (Iron): 1000 mg, Mg (Magnesium): 55 gm, Se (Selenium): 3 mg, Na (Sodium): 45 gm.

However maximum values of carbohydrate (19%) and crude fiber (1%) were observed in fish fed at 3 and 0 mg Kg^{-1} Cr-nano level based diets, respectively. Minimum ash contents (7%) and moisture contents (5%) were recorded in fish fed at 1.5 mg Kg^{-1} Cr-nano level based diet that were significantly ($p<0.05$)

different from the fish which fed at other Cr-nano supplemented diets and without supplemented Sunflower meal based diet (control diet). However, maximum moisture (7%) and ash (10%) contents were found in the fish fed at control diet and 3 mg Kg^{-1} Cr-nano level based diets (respectively).

These findings indicate that a decline in carbohydrate, crude fiber, moisture, and ash contents was observed generally when fish fed on Cr-nano supplemented test diets. Lowest values for these parameters were noted in the fish fed at 1.5 and 2 mg Kg^{-1} Cr-nano diets. So Cr-nano supplementation is necessary for higher nutrient (CP and EE) contents in feed to improved fish growth performance.

It was found that 2 and 1.5 mg Kg^{-1} Cr-nano supplementation in diet is the best level for the maximum deposition of protein and lipids respectively in fish; these nutrients are essential for fish performance. Moreover almost lowest carbohydrate, ash, crude fiber as well as moisture contents were observed in *C. mrigala* fed 1.5 and 2 mg Kg^{-1} Cr-nano diets.

Table 3. Proximate composition (%) of *C. mrigala* carcass fed graded levels of Cr -nano supplemented Sunflower meal based diets.

Diets	Cr -nano (mg kg^{-1})	Protein	Fat	Ash	Moisture	Crude fibre	Carbohydrate
Test Diet –I (Control diet)	0	54.45^{e}	9.23^{d}	9.34^{ab}	7.10^{a}	1.26^{a}	18.62^{a}
Test Diet –II	0.5	56.81^{d}	11.97^{c}	9.53^{a}	6.34^{b}	1.10^{a}	14.26^{b}
Test Diet –III	1	57.04^{cd}	13.01^{ab}	8.26^{cd}	7.14^{a}	1.06^{a}	13.48^{bc}
Test Diet –IV	1.5	57.69^{c}	13.82^{a}	7.61^{d}	5.17^{c}	1.02^{a}	14.70^{b}
Test Diet –V	2	59.34^{a}	12.82^{b}	8.62^{bc}	5.36^{c}	1.19^{a}	12.67^{c}
Test Diet –VI	2.5	58.47^{b}	13.64^{ab}	7.61^{d}	5.27^{c}	1.02^{a}	14.00^{bc}
Test Diet –VII	3	53.87^{e}	9.06^{d}	9.62^{a}	7.01^{ab}	1.24^{a}	19.21^{a}
PSE		0.136951	0.170968	0.161285	0.152034	0.054102	0.32808
P Value		0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0226*	0.0000 ***

Means within columns having different superscripts are significantly different at $p < 0.05$

Data are means of three replicates

PSE = pooled SE = √MSE/n (where MSE = Mean-Squared Error).

Discussion

The results of current study proved that nano particles supplementation is very important to improve the carcass composition of *C. mrigala* fingerlings. Maximum values of CP and EE were observed in fish fed at 1.5-2 mg Kg^{-1} Cr-nano diet. These results are similar to the findings of Srinivasan *et al.* (2016) who found that supplementation of Fe_2O_3 NPs significantly improved carcass parameters as compared to control diets in giant fresh water prawn. Wang and Xu (2004) reported that supplementation of Cr-Nanoparticles has beneficial effects on carcass composition, pork quality and individual skeletal muscle weight. Dietary Cr-Nano supplementation increased (p<0.05) the lean ratio of carcass of pigs by 14.06%. This improvement is due to the special metabolism pathway and deposition mechanism of NPs in carps due to which soluble proteins can interact with nanoparticles to form halo (crona). Nano-protein cronas can interfere with protein folding and can enhance protein cross linking (Zhou *et al.,* 2009; Onuegbu *et al.,* 2018). When concentration of NPs crosses the optimum levels then feed starts to lose palatability which may be the possible reason of decrease of carcass parameters on higher levels of supplementation (Onuegbu *et al.*, 2018). Cr is involved in protein metabolism. It also plays an important role as an integral component of the glucose tolerance factor, which improves the potential the action of insulin and regulates glucose metabolism (Sirriat *et al.,* 2012). On the other hand, Ashuria *et al.* (2015) reported that proximate composition of fish was not affected by the dietary treatment after 8-weeks of culture, indicating that the carp muscle proximate composition is not sensitive to dietary Se treatments. Similar observation was also reported by Le *et al.* (2013) for juvenile yellowtail king fish. Wang *et al.* (2015) studied the effects of Cu-NPs and soluble Cu on carcass composition of juvenile *Epinephelus coioides*. The fish were exposed in triplicate to control, 20 or 100μg CuL^{-1} as either copper sulphate ($CuSO_4$) or Cu-NPs for 25 days. With an increase in $CuSO_4$ and Cu-NPs dose, crude protein and crude lipid decreased while ash and moisture increased ultimately causing decrease in growth performance of fish.

Conclusion

It was concluded from the results of current study that supplementation of Cr NPs at the rate of 1.5-2 mg Kg^{-1} in sunflower meal based diet improves the body composition of *C. mrigala* fingerlings.

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