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Efficacy of Nano-Cr Particles Supplemented Sunflower Meal Based Diets on Growth Performance, Digestibility and Hematology of *Catla catla* Fingerlings

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Authors' Contribution
SMH planned and supervised the research. NA conducted the research. MAS and NA wrote the manuscript. AR and MAS helped in chemical analysis. ML helped in statistical analysis. MZH conducted feeding trial. MU helped in fish feed formulation. HHS collected fish feces.

Key words
Nano- Cr particles, growth, nutrient digestibility, hematology, *C. catla*

ABSTRACT

Molecules at nano size possesses novel properties regarding absorbance and surface activity, so the aim of research was to estimate the impact of Cr nanoparticles on growth performance, nutrient digestibility and hematological parameters of *C. catla* fingerlings fed nano- Cr particles supplemented sunflower meal based diets. The experiment was consisted on seven test diets on the basis of supplementation of nano Cr graded levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg Kg^{-1}). Chromic oxide was used as an inert marker. Fingerlings were fed at the rate of 5% of their live wet weight. Maximum improvement in the growth performance (weight gain 197%, FCR 1.49, SGR 1.21 and survival 100%), nutrient digestibility (crude protein 71% and gross energy 69%) and hematological parameters (WBCs 7.87 ×103mm^{-3}, RBCs 3.04 ×106mm^{-3} and Platelets 67) were observed in the fingerlings fed with test diet supplemented with 2 mg kg^{-1} nano Cr while crude fat digestibility (79%) was found maximum at test diet supplemented with 1.5 mg kg^{-1} of nano Cr which were significantly different from fish fed with control and other test diets. It was concluded that the supplementation of Nano-Cr particles at the rate of 2 mg kg^{-1} is the best one to improve growth performance, nutrient digestibility and hematological parameters of *C. catla* fingerlings fed sunflower meal based diets.

INTRODUCTION

The major goal for fish industry is to develop cost effective fish feed (Baruah *et al*., 2004). Fishmeal has been used for essential nutrients and growth factors in fish feed (Zhou *et al*., 2004). Due to high cost, uneven supply and increasing demand of fish meal it has become necessary to search for alternative protein sources (Pham *et al*., 2008; Lech and Reigh, 2012). In fish culture industry, only feed accounts more than 50% of total cost of fish culture (Essa *et al*., 2004). Positive effects in terms of fish growth have been reported by using plant meal in diet (Hussain *et al*., 2011; Shahzad *et al*., 2018). Plant by-products are being used as alternative protein sources because they are cheaper, environment friendly and easily available as compared to fish meal (Gatlin *et al*., 2007; Dalsgaard *et al*., 2009). Sunflower meal contains about 45-48% crude protein (Mushtaq *et al*., 2006) and is being used in feed formulations as it contains endogenous proteolytic enzymes to digest proteins for fish (Kocher *et al*., 2000). In Pakistan this crop has lowest cost among plant protein sources (Khan *et al*., 2006). It is important to formulate nutritionally balanced and highly palatable feed which results in maximum growth of fish (Afzal *et al*., 2004; Tahir *et al*., 2008).

Nanotechnology is developing continuously as is being applied with a high potential for the betterment of livestock production and animals in general. In nanotechnology structures under 100nm in size are being used, which is 1000 times narrower than the diameter of a human hair. The nanoparticles increase the bioavailability of nutrients and carry them via the gastrointestinal tract (GIT) into the bloodstream. Nanoparticles also improve the bioavailability of ώ- 3 fatty acids, natural antioxidants and trace minerals (Bunglavan *et al*., 2014). Nanoparticles

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may increase the bioavailability of nutrients. Nano form of supplementation increases the surface leading increase in absorption and utilization of minerals (Vijayakumar and Balakrishnan, 2014). Nanoparticles exhibit novel characteristics, such as great specific surface area, high surface activity, high catalytic efficiency and the character of low toxicity (Pelyhe and Miklós, 2013). Nanomaterials possess greater bioavailability than larger molecules (Albrecht *et al*., 2006; Wang *et al*., 2007). In feed industry nano-particles are being used in feed processing and dietary supplementations (Powell *et al*., 2010).

Nanotechnology tools are being used in aquaculture industry for rapid disease detection, and targeted delivery of nutrients (Ashraf *et al*., 2011). Supplementation of nanoparticles can improve the absorption of minerals. Chromium (Cr) is an essential trace element for humans and animal's health. It plays important role in insulin receptor activation. Cr is also involved in the metabolism of biological molecules like carbohydrates, nucleic acids, lipids and proteins whereas its deficiency results in growth retardation (Wang and Xu, 2004). Cr nano increases immunoglobulin contents in blood plasma (Wang *et al*., 2007). A key factor which affects the digestion and absorption of Cr in intestinal tract is particle size and nature of the polymer (Wang and Xu, 2004).

Catla catla is one of the most important fish with maximum market demand and it contributes along with *Labeo rohita* and *Cirrhinus mrigala* about 67% of total freshwater fish production in South India (Krishnaveni *et al*., 2013). The major objectives of this study were to evaluate the effects of Cr NPs supplementation on growth performance, nutrient digestibility and hematological parameters of *C. catla* fingerlings.

MATERIALS AND METHODS

C. catla fingerlings were purchased from Government Fish Seed Hatchery Faisalabad. Fingerlings were acclimatized for 15 days in Fish Nutrition Laboratory, GCUF and were fed on the basal diet once in a day (Allan and Rowland, 1992).

Analysis of feed ingredients and Cr-nano particles

The feed ingredients were analyzed by following standard methods (AOAC, 1995). Cr-nano particles were purchased from market (Sigma-Aldrich), to confirm their pure crystalline structure and size, they were analyzed by XRD and TEM (TEM-JEOL2100-20171206), respectively (Ramamurthy *et al*., 2013; Iqbal *et al*., 2014).

Formation of pellets

All the feed ingredients were ground until they passed through 0.5mm sized sieve. All ingredients were mixed for 5 min, fish oil was gradually added (Table I). Finally, water was added slowly to make suitable dough and pellets were formulated thereafter with the help of pelleting machine by following Lovell (1989).

Preparation of NP stock solution

Preparation and confirmation of stock solutions of NPs was carried out according to Federici *et al*. (2007). Stock solution of 100% pure NPs dry powder was made by sonication method (for 6-8 h) and from these stock solutions further dilutions were made to ensure our required levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg Kg^{-1}) of Cr NPs.

Addition of NPs to basal diets

The diluted Cr solutions were sonicated further for 15 min just before spraying on basal diets according to Ramsden *et al*. (2009). One-Kg feed was placed in a commercial food mixer and gradually sprayed with the appropriate dilution. The seven test diets were formulated by mixing graded levels (0, 0.5, 1, 1.5, 2, 2.5 and 3 mg Kg^{-1}) of nano Cr. NPs immediately coated the feed pellets. The feed pellets were allowed to dry then ultimately were stored in air tight containers for further use.

Feeding protocol and sample collection

C. catla fingerlings were fed for 90 days on prescribed diets mentioned above at the rate of 5% of their live wet weight. Three replicates were used for each diet and 15 fingerlings were stocked in each replicate. Their feces were collected by alternative opening and closing of valve-I and valve-II following Hussain *et al*. (2018).

Chemical analysis of feed and feces

Feed ingredients, experimental diets and samples of feces were homogenized and then subsequently analyzed by standard methods (AOAC, 1995). Protein by micro-Kjeldahl apparatus, crude fat by ether extraction method, crude fiber by loss on ignition and gross energy was determined by oxygen bomb calorimeter (Table II).

Chromic oxide contents in diets and feces were estimated by following Divakaran *et al*. (2002) using UV-VIS 2001 spectrophotometer at 370 nm absorbance.

Growth study

Growth performance of fingerlings was evaluated by following standard methods as defined by Hussain *et al*. (2015).

$$\text{Weight gain \%} = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Initial weight}} \times 100$$

$$FCR = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$SGR\% = \frac{(\ln.\text{final wt. of fish} - \ln.\text{initial wt. of fish})}{\text{Trial day}} \times 100$$

Nutrient digestibility

Apparent nutrient digestibility coefficients (ADC) for experimental diets were calculated by the formula reported in NRC (1993).

$$ADC\ (\%) = 100 - 100 \times \frac{\%\ \text{marker in diet} \times \%\ \text{nutrient in feces}}{\%\ \text{marker in feces} \times \%\ \text{nutrient in diet}}$$

Study of hematological parameters

Hematological parameters were evaluated following Peake (1998) and Coyle *et al.* (2004). Hematocrit was determined using capillary tubes (Brown, 1980). RBCs and WBCs were counted with a haemo-cytometer (Blaxhall and Daisley, 1973). Hb (Hemoglobin) concentration was determined as described by (Blaxhall and Daisley, 1973). To calculate mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) following formulae were used:

$$MCHC = Hb/PCV \times 100$$

$$MCH = PCV/RBC \times 10$$

$$MCV = Hb/RBC \times 10$$

Statistical analysis

One-way analysis of variance was applied to data of haematology, digestibility and growth of fish (Steel *et al.*, 1996). Tukey's Honesty Significant Difference Test ($p<0.05$) was used to compare the differences among various levels (Snedecor and Cochran, 1991). For statistical analysis CoStat Computer Package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

RESULTS

Results of morphological analysis of the Cr-NPs by using Transmission electron microscope (TEM) are shown in (Fig. 1). TEM confirms the shape and size of Cr NPs, as magnified form depicted in (Fig. 1a) and normal TEM form in Figure (Fig. 1b). In these samples, the scale bar was set to 10 nm in case of magnified TEM image and 100 nm of scale bar in terms of normal form TEM image. TEM image justify the rods form of Cr NPs with about 25-30 nm diameter and about 40-70nm in length almost homogenous structure format. The above results of TEM confirm that Cr NPs used in experimental diets of our study contain size less than 100nm about 25-30 nm. It confirms that they are pure nano particles in their nature.

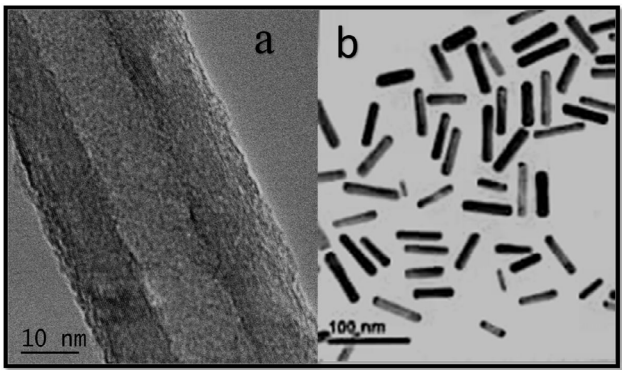

Fig. 1. Transmission Electron Microscopic (TEM) view of chromium nano rods; 10 nm scale bar with magnified form (b) 100 nm Scale bar.

Table I.- Chemical composition (%) of feed ingredients.

Ingredients	Fish meal	Rice polish	Wheat flour	Sun-flower
Dry matter (%)	91.67	94.06	92.4	93.80
Crude Protein (%)	49.03	11.87	09.73	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	09.96
Gross Energy (kcal/g)	2.49	3.41	3.06	3.64
Carbohydrates	19.66	52.21	82.21	43.6

The crystal structure and the phase composition of chromium nanoparticles were confirmed by using X-Ray Diffraction (XRD) techniques shown in (Fig. 2). The XRD pattern confirms very clearly that the sample is nano-crystalline in nature as it matches very well with that of the standard chromium powder of chromium nano-particles. Growth parameters such as weight gain (13.47 g), weight gain% (197 %), specific growth rate (1.21) and survival (100%) were observed maximum at 2 mg kg^{-1} nano Cr as shown in Table III. Second higher value of growth parameters (weight gain 13.08 g, weight gain% 191 % and SGR 1.19) were observed in the fingerlings fed on test diet VI (2.5 mg kg^{-1} nano Cr). Lowest/best FCR (1.49) was observed in the fingerlings fed at 2 mg kg^{-1} nano Cr followed by (1.57) the fish fed at 2.5 mg Kg^{-1} nano Cr. It was observed that these values were statistically different (weight gain 11.49 g, weight gain% 168 %, SGR 1.1 andsurvival 96 %) from the fish fed on control diet. Lowest weight gain (9.64 g), weight gain% (141 %) and SGR 0.98) were noted in the fingerlings that were fed on test diet VII (3 mg kg^{-1} nano Cr).

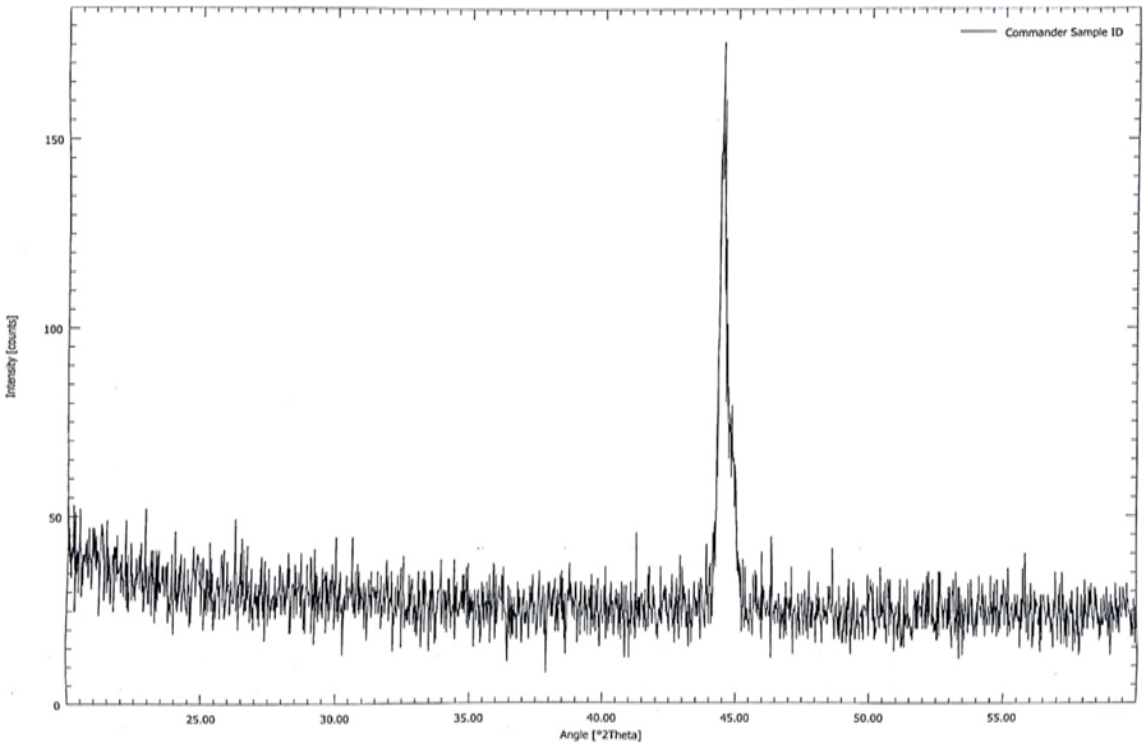

Fig. 2. X-Ray Diffraction (XRD) view of Chromium Nano-rods.

Table II.- Ingredients composition (%) oilseed 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	3.0
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.

Table III. Growth performance of *C. catla* fingerlings fed graded levels of Cr-nano supplemented Sunflower meal based diets.

Diets	Cr -nano (mg kg^{-1})	IW (g)	FW (g)	WG (g)	WG (%)	FI (g)	WG ($fish^{-1}$ day^{-1})g	FCR	SGR	Survival %
Test Diet –I (Control diet)	0	6.83	18.32 ^{c}	11.49 ^{d}	168.11 ^{d}	0.23 ^{a}	0.13 ^{d}	1.83 ^{b}	1.10 ^{d}	95.56 ^{a}
Test Diet –II	0.50	6.83	18.91 ^{bc}	12.08 ^{cd}	176.95 ^{cd}	0.23 ^{a}	0.13 ^{cd}	1.72 ^{c}	1.13 ^{cd}	97.78 ^{a}
Test Diet –III	1.00	6.82	19.28 ^{abc}	12.46 ^{bc}	182.66 ^{bc}	0.23 ^{a}	0.14 ^{bc}	1.64 ^{cd}	1.15 ^{bc}	95.56 ^{a}
Test Diet –IV	1.50	6.83	19.76 ^{ab}	12.93 ^{ab}	189.22 ^{ab}	0.23 ^{a}	0.14 ^{ab}	1.59 ^{d}	1.18 ^{ab}	97.78 ^{a}
Test Diet –V	2.00	6.83	20.29 ^{a}	13.47 ^{a}	197.27 ^{a}	0.22 ^{ab}	0.15 ^{a}	1.49 ^{de}	1.21 ^{a}	100.00 ^{a}
Test Diet –VI	2.50	6.86	19.94 ^{ab}	13.08 ^{ab}	190.72 ^{ab}	0.23 ^{a}	0.15 ^{ab}	1.57 ^{e}	1.19 ^{ab}	100.00 ^{a}
Test Diet –VII	3.00	6.85	16.49 ^{d}	9.64 ^{e}	140.65 ^{e}	0.21 ^{b}	0.11 ^{e}	1.97 ^{a}	0.98 ^{e}	97.78 ^{a}
*PSE		0.077919	0.232215	0.174206	2.049886	0.001936	0.003124	0.020275	0.008075	1.680682
P Value		.9998ns	.0000***	.000***	.000***	.000***	.0031**	.000***	.000 ***	.0991 ns

Means within columns having different superscripts are significantly different at P< 0.05. Data are means of three replicates. IW, Initial Weight; FW, Final Weight; WG, Weight gain; FI, Feed Intake; SGR, Specific Growth Rate; FCR, Feed Conversion Ratio; *PSE, pooled; SE= √MSE/n (where MSE = mean-squared error).

Table IV. Percentage of nutrients in test diets of *C. catla* fingerlings fed graded levels of Cr -nano supplemented Sunflower meal based diets.

Diets	Cr -nano (mg kg^{-1})	CP (%) in diet	EE (%) in diet	GE (kcal g^{-1}) in diet
Test Diet –I (Control diet)	0	31.40	6.94	3.87
Test Diet –II	0.50	31.49	6.94	3.87
Test Diet –III	1.00	31.39	6.92	3.86
Test Diet –IV	1.50	31.35	6.94	3.87
Test Diet –V	2.00	31.43	6.92	3.88
Test Diet –VI	2.50	31.36	6.92	3.86
Test Diet –VII	3.00	31.20	6.91	3.86
*PSE		0.172171	0.0290322	0.035367
P Value		.9387 ns	.9642 ns	.9999 ns

Means within columns having different superscripts are significantly different at P< 0.05. Data are means of three replicates. CP, Crude protein; EE, ether extract (crude fat), GE, gross energy. *PSE, pooled SE = √MSE/n (where MSE = mean-squared error).

The percentages of nutrients in feed, feces and their digestibility are given in Tables IV, V and VI, respectively. Results showed that all the respective nutrients were statistically similar in all the test diets fed to fingerlings but significantly different in feces when they fed on different concentrations based diets. Lowest nutrients were discharged through feces when fish fed test diet-IV (2 mg Kg^{-1}) whereas maximum nutrients were released in water when they fed with 3 mg Kg^{-1} nano Cr based diet. The ADC% of nutrients (CP 71% and GE 69%) were observed at their highest in fingerlings fed 2 mg kg^{-1} of nano- Cr while highest ADC% of crude fat 79% was observed at 1.5 mg kg^{-1} of nano- Cr, respectively. These values were significantly different from that of control group (CP: 58%, EE: 55% and GE: 55%). On the other hand lowest values (EE: 54%, GE 50% and CP: 56%) were noted at 3 and 0 mg Kg^{-1}, respectively.

Supplementation of Cr-nano improved the hematological indices of *C. catla*. Results showed that the best values of RBCs (3.04 $\times 10^{6} mm^{-3}$), WBCs (7.87$\times 10^{3} mm^{-3}$), Hb (8.56 g/100ml), MCV (189.60 fl), PCV (25.08), PLT (67.23), MCH (51.31 pg) and MCHC (33.95 %) were observed in fish that fed on Cr-nano based diet supplemented at 2 mg Kg^{-1} Cr-nano particles (Table VII). The least values of RBCs (1.30 $\times 10^{6} mm^{-3}$), WBCs (6.70 $\times 10^{3} mm^{-3}$), Hb (6.13 g/100ml), MCV (90.67), PCV (21.54), PLT (53.83), MCH (35.38 pg) and MCHC (25.63 %) were observed in fish fed at 3 mg kg^{-1} Cr-nano level based diet. While in control group, these values were: RBCs (2.04 $\times 10^{6} mm^{-3}$), WBCs (7.05 $\times 10^{3} mm^{-3}$), Hb (7.09 g/100ml), MCV (100.06), PCV (23.04), PLT (57.21), MCH (40 pg) and MCHC (28 %). So it is clear that these parameters started to improve from 0.5 to 2 mg Kg^{-1} Cr-nano level based diets and thereafter started to decrease on higher (2.5 and 3 mg Kg^{-1} Cr-nano level) supplementation in sunflower meal based diets.

Overall it was observed that growth, digestibility and hematological indices were started to improve from 0.5 up to 2 mg Kg^{-1} Cr-nano level based diet and started to decrease at further increase of Cr-nano supplementation.

Table V. Percentage of nutrients in feces of *C. catla* fingerlings fed graded levels of Cr -nano supplemented sunflower meal based diets.

Diets	Cr-nano (mg kg^{-1})	CP (%) in feces	EE (%) in feces	GE (kcal g^{-1}) in feces
Test Diet –I (Control diet)	0	14.61^{ab}	3.42^{a}	1.90^{b}
Test Diet –II	0.5	15.20^{a}	2.84^{a}	2.01^{ab}
Test Diet –III	1	13.49^{bc}	2.14^{a}	1.82^{b}
Test Diet –IV	1.5	11.72^{d}	1.60^{a}	1.57^{c}
Test Diet –V	2	10.11^{e}	2.07^{a}	1.33^{d}
Test Diet –VI	2.5	13.08^{cd}	2.78^{a}	1.81^{b}
Test Diet –VII	3	15.23^{a}	3.54^{a}	2.16^{a}
*PSE		0.314822	0.0515167	0.045513
P Value		0.0000***	0.0000***	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).

Table VI. Percentage of nutrients digestibility of *C. catla* fingerlings fed graded levels of Cr -nano supplemented sunflower meal based diets.

Diets	Cr-nano (mg kg^{-1})	CP (%) digestibility	EE (%) digestibility	GE(%) digestibility
Test Diet–I (Control diet)	0	57.53^{e}	55.08^{ab}	55.30^{e}
Test Diet –II	0.5	55.63^{f}	62.40^{ab}	52.20^{f}
Test Diet –III	1	60.07^{d}	71.27^{a}	56.24^{d}
Test Diet –IV	1.5	66.25^{b}	79.13^{a}	63.38^{b}
Test Diet –V	2	71.15^{a}	73.17^{a}	69.23^{a}
Test Diet –VI	2.5	62.07^{c}	63.42^{ab}	57.35^{c}
Test Diet –VII	3	56.50^{f}	54.41^{ab}	50.09^{g}
*PSE		0.204686	0.213874886	0.192056
P Value		.0000 ***	.0000 ***	.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).

Table VII. Hematological parameters of *C. catla* fingerlings fed graded levels of Cr -nano supplemented Sunflower meal based diets.

Diets	Cr-nano (mg kg^{-1})	RBC (106mm^{-3})	WBC (103mm^{-3})	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
Test Diet –I (Control diet)	0	2.04^{d}	7.05^{cd}	57.21^{d}	7.09^{b}	23.04^{c}	28.16^{de}	40.00^{d}	100.06^{f}
Test Diet –II	0.5	2.64^{c}	7.27^{bc}	61.76^{c}	7.82^{ab}	23.20^{c}	28.55^{cde}	36.10^{e}	125.25^{e}
Test Diet –III	1	2.69^{bc}	7.63^{ab}	64.58^{b}	8.03^{a}	24.18^{ab}	29.35^{bcd}	40.06^{d}	167.18^{d}
Test Diet –IV	1.5	2.73^{bc}	7.69^{ab}	63.90^{b}	8.15^{a}	24.22^{ab}	31.83^{ab}	42.82^{c}	170.83^{c}
Test Diet –V	2	3.04^{a}	7.87^{a}	67.23^{a}	8.56^{a}	25.08^{a}	33.95^{a}	51.31^{a}	189.60^{a}
Test Diet –VI	2.5	2.85^{ab}	7.74^{ab}	63.90^{b}	8.32^{a}	23.95^{bc}	31.27^{abc}	45.89^{b}	186.84^{b}
Test Diet –VII	3	1.30^{e}	6.70^{d}	53.83^{e}	6.13^{c}	21.54^{d}	25.63^{e}	35.38^{e}	90.67^{g}
*PSE		0.04080523	0.105055	0.190013	0.15853	0.202139	0.607528	0.250574	0.252681
P Value		.0000***	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***

Means within columns having different superscripts are significantly different at P< 0.05. Data are means of three replicates. WBC, White blood cell; RBC, Red Blood Cell; PCV, Packed cell volume; Hb, hemoglobin concentration; PLT, Platelet; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration. *PSE = pooled SE = √MSE/n (where MSE = mean-squared error).

DISCUSSION

All the growth parameters such as weight gain, weight gain %, FCR, SGR and survival rate were improved by the supplementation of Cr nanoparticles. These findings aresupported by Ashouri *et al*. (2015). They used Nano-Se at the levels of 0, 0.5, 1 and 2 mg Kg^{-1} for 8 weeks and found that all levels were significantly higher than that of control one in all parameters except survival which was 100% in all four treatments. They also observed that weight gain (96%) of fish remained highest at 1 mg Kg^{-1} of nano-Se significantly different from basal diet (60%). Faiz *et al*. (2015) also presented similar results with current findings. They found 85% difference in WG of grass carp juvenile when they fed nanoparticles of zinc oxide. They used two levels that of 30mg Kg^{-1} and 60mg Kg^{-1} while best results were at the first one; 30mg Kg^{-1}. Zhou *et al*. (2009) also found a significant improvement

in growth parameters such as weight gain (10.88g), FCR (1.63) and relative gain rate (77.52) in crucian carp fed on nano-Se (0.5mg Kg^{-1}) as compared to control one (WG 7.62g, FCR 1.73 and RGR 52.75%). Srinivasan *et al*. (2016) found that all the growth parameters (WG 1.61g, SGR 1.30%, FCR 0.86) and survival rate (90.83%) of giant fresh water prawn were significantly improved when they were fed on iron oxide nanoparticles as compared to that of control diet (WG 0.74g, SGR 0.99%, FCR 1.34 and SR 80.03%). The above said improved results were found at Fe_2O_3 nano particles supplementation at the rate of 20 mg/Kg while the others levels were 10, 30, 40 and 50 mg/Kg. These differences in results for growth indices may be due to the many factors such as processing methods of feed, types of feed ingredients used, pH of stomach and methods used for feed drying. Bagheri *et al*. (2015) found that the use of nano-Se at the level of 0.5 mg Kg^{-1} caused the best feed conversion ratio (1.67) in chicken while FCR of control group was 1.73. Also, the level of 0.5 mg/kg Nano-Se showed the most average daily weight gain (52g) in comparison to the control diet (45g) at the end of experimental period ($p<0.05$).

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 Ramsden *et al*. (2009) observed no effect on growth of rainbow trout when exposed to TiO_2 nanoparticles. Wang *et al*. (2015) reported that the growth was decreased with increasing doses of $CuSO_4$ or Cu-NPs. The fish (*Epinephelus coioides*) were divided in three groups: control, exposed to 20 and 100 g CuL^{-1} $CuSO_4$ or Cu-NPs for twenty five days. Dobrochna *et al*. (2018) reported that rainbow trout fingerlings treated by AgNPs and CuNPs groups showed lower growth rate as compared to the control one.

Current research suggests that nano particles are very important for useful and proper nutrient digestibility of *C. catla* fingerlings. Similarly Kumari *et al*. (2013) reported that supplementation of nano encapsulated trypsin improved digestibility of *L. rohita*. They used two levels (0.01% and 0.02%) of nano encapsulated trypsin and found better results on 0.01%. Eguia *et al*. (2009) also observed a significant increase in digestibility of all nutrients when piglets were treated with 50mg Kg^{-1} CuNPs supplemented diet as compared to control one. However contrary to our results there was no significant difference of crude protein digestibility of both groups. On the other hand Wang *et al*. (2015) found that crude protein and crude lipid decreased with an increase in $CuSO_4$ and Cu-NPs dose, ultimately causing decrease in growth performance of fish.

Hematological parameters are very important to study out the proper health of fish because the blood provides information about the fish physiology. In present study we found that all of the important hematological parameters like WBCs, RBCs, Platelets, MCV, PCV, Hb, MCH and MCHC were observed at their highest level in the fish fed with supplementation of 2 mg kg^{-1} of nano particles. These results are coinciding with the findings of Behera *et al*. (2014) that nano-Fe improves the hematological indices of *L. rohita*. Similar findings were reported by Prochorov *et al*. (2011). Bagheri *et al*. (2015) reported that Nano-Se supplementation of chicken diets was effective in increasing the hematological parameters of chicken. Khan *et al*. (2016) reported an improvement in blood indices of fish fed the Se-NP-supplemented diet (0.68 Se-NPs mg Kg^{-1}) compared to the control group (0 Se-NPs mg Kg^{-1}) of fish. Similarly, Khalafalla *et al*. (2011), Sadeghian *et al*. (2012) and Le *et al*. (2013) found that Se-nano improved the immunity of fish and RBC count of the fish which is also parallel to our findings. The reason behind the improvement of hematological parameters may be the strong antioxidant property of nano particles which provide stability and integrity of cells inside the animal's body and protect them from hemolysis, however, at a high levels, it can become toxic and produce harmful effects (Khan *et al*., 2016). The hematopoietic effects of Cr could be related to the protection of cell membrane and intracellular organelles by the antioxidant effects of NPs and thus increase the life span of RBC and leukocytes (Alimohamady *et al*., 2013).

CONCLUSION

Our research work provides sufficient evidence that supplementation of the Cr nano particles are helpful for the proper growth performance, nutrients digestibility and improvement of hematological parameters of *C. catla* fingerlings fed sunflower meal based diets. It was also

concluded that 2 mg Kg^{-1} supplementation levels of Cr-nano particles is the optimum level for the improvement of all above said factors and higher supplementations could not cause further improvement.

Statement of conflict of interest

The authors declare that there is no conflict of interests.

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