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CONTENTS

Dietary supplementation of formulated fish-specific mineral mixtures improved the growth, nutrient composition and health status of *Cyprinus carpio* fingerlings

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ABSTRACT: The goal of the current study was to determine how a mineral mixture specifically designed for fish affected their growth, nutritional makeup, and overall health of *Cyprinus carpio*. The experiment consists of five treatments (T1-T5, in triplicate); the basal diet used for all the treatments consisted of rice bran (49-50%), mustard oil (48.5-49%), mineral mixture (0-2%) and salt (0.5%); the positive control with commercially available mineral mixture (T1) and the negative control without any mineral mixture (T2) and three (T3-T5) treatments supplemented with formulated mineral mixture (as per ICAR, 2013 recommendations) at three inclusion levels of 1%, 1.5% and 2%, respectively. All the major water quality parameters were within the acceptable limits. When compared to the non-supplemented group (T2), the growth performance of *C. carpio* fingerlings, including final weight, weight gain, and specific growth rate, has been significantly ($P<0.05$) improved by the developed mineral supplementation. Additionally, the T4 group's feed conversion ratio and protein efficiency ratio were shown to be much improved ($p<0.05$). Moreover, the total protein and fat content of the flesh were significantly ($p<0.05$) boosted by the mineral mixture. Haemoglobin, haematocrit, total erythrocyte count, total leucocyte count, mean corpuscular volume, mean corpuscular haemoglobin, and mean cell haemoglobin concentration were considered as responsible parameters for haematological study and were also significantly ($P<0.05$) improved and best recorded at 1.5% (T4) mineral mixture compared to the non-supplemented group. The information gathered for this study will help a number of stakeholders, including fish farmers, entrepreneurs, and the feed sector, increase fish production and productivity in a sustainable manner.

Keywords: *Cyprinus carpio*, growth, health, mineral mixture, proximate composition

For their essential physiological and biochemical processes as well as to sustain their regular life cycles, all aquatic species need minerals. Unlike other vertebrates, fish can live in a wide range of salt levels and take up minerals from their food and the water around them. Fish tissues contain the majority of the vital minerals needed by animals and other vertebrates (Suttle, 2010). It has been established that fish require specific trace elements (cobalt, copper, iodine, iron, manganese selenium, and zinc) as well as macrominerals (calcium, phosphorus, magnesium, sodium, potassium, and chloride) (NRC, 1993; 2011; Musharraf and Khan, 2022). Fish have not been found to contain other trace elements (arsenic, boron, chromium, fluorine, nickel, lithium, lead, molybdenum, silicon, and vanadium) that are thought to be necessary for humans and animals due to the impairment of particular physiological activities (Lall and Kaushik, 2021).

More than 95% of total fish production comes from freshwater aquaculture (FAO, 2020).Aquaculture is a feed based industry where fish feed alone constitutes 60% of operational cost (Paul and Mohanty, 2002). Over 85% of the fish produced in India's freshwater aquaculture industry are carps. Fish need minerals for several aspects of their lives. In order to absorb and retain minerals from their food and water, aquatic animals have special physiological processes. There are significant gaps in our understanding of the physiological roles, trace element requirements, and feed ingredient bioavailability, and research and development in the field of mineral nutrition for farmed fish has been rather modest. For certain fish species, quantitative dietary needs have been documented for six trace minerals (zinc, iron, copper, manganese, iodine, and selenium) and three macro-elements (calcium, phosphorus, and magnesium). Reduced bone mineralisation, anorexia, lens cataracts (zinc), skeletal abnormalities (phosphorus, magnesium, zinc), fin erosion (copper, zinc), nephrocalcinosis

(magnesium deficiency, selenium toxicity), thyroid hyperplasia (iodine), muscular dystrophy (selenium), and hypochromic microcytic anaemia (iron) are all indicators of mineral deficiencies in fish. In order for aquatic species to maintain their homeostasis through enhanced absorption or excretion, a delicate balance between mineral deficiency and toxicity is essential. Excessive intake of minerals from either diet or gill uptake causes toxicity. Feed formulation must take extra care since the release of minerals from undigested or uneaten feed and from urine excretion might eutrophicate natural streams (Lall, 2002). A substantial amount of minerals, frequently more than the projected requirement, are provided by the animal and plant feed ingredients utilised in the creation of artificial feed (Gatlin and Wilson, 1986; Storebakken *et al*., 2000). Minerals are typically added to carp feed at a rate of 1% to 2% (Mishra and Mukhopadhyay, 1996; Datta and Kaviraj, 2003). The needs of various species, the availability of the minerals in the medium and natural food, and their bioavailability are not given much consideration. These fish species get different amounts of minerals from natural sources depending on how they feed. For several of these fish species, live plankton is a significant source of minerals. It has been discovered that an exogenous source of plankton improves carp growth (Chakrabarti and Jana, 1992). According to Lubzens *et al*. (1984), giving *Cyprinus carpio* larvae rotifers in addition to artificial food greatly increased their growth rate and survival. There isn't a fish-specific mineral in the local market. Furthermore, the common mineral mixtures on the market are of poor quality and are also expensive. Thus, the goal of the current study was to standardise the dosage and effectiveness of a mineral mixture specifically designed for *Cyprinus carpio* fingerlings based on ICAR, 2013 recommendations regarding carp mineral requirements according to Guru Angad Dev Veterinary and Animal Sciences University (GADVASU) scientists.

MATERIALS AND METHODS

Experimental design

The study was conducted at the Fish Farm of College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, in outdoor FRP pools of 1.5 x 1.0 x 0.75 meters. There were five treatments in which T1 and T2 were considered positive and negative control respectively in the experiment, each with triplicates.

Water quality parameters

According to the procedures of APHA (2012), water samples were taken every two weeks in the morning at 10 A.M. for the analysis of physico-chemical parameters, including temperature, pH, dissolve oxygen (DO), total alkalinity (TA), nitrate, nitrite, and ammonia.

Growth parameters

Growth characteristics in terms of total length gain (TLG), net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) and condition factor (K) of fish for each treatment were computed (Halver, 1957).

Stocking of fingerlings

In order to replicate natural conditions, a three-inch thick layer of soil was placed at the bottom of each pool and the tank preparation was done as per the standard methods. After 15 days of fertilization all the fifteen tanks were stocked with 15 fingerlings (11 - 12 cm and 15 – 16 gm) of *Cyprinus carpio*/ FRP pool, after exposing the fish to a dip treatment with 5 ppm of $KMnO_4$ solution.

Preparation of experimental diet

De-oiled rice bran (49–50%), mustard cake (48.5–49%), mineral mixture (0–2%), and salt (0.5%) were used to make the basal diet which was used for all the treatments. Adequate water was added, and 2% carboxymethyl cellulose (CMC) was added as a binder (above other ingredients) to the produced diet (Table 1). Five treatments (T1-T5 in triplicate), positive control with commercially available mineral mixture (T1) and negative control without any mineral mixture (T2) and three (T3-T5) treatments supplemented with formulated mineral mixture (as per ICAR, 2013 recommendations and contain elements Ca, P, Mg, Cu, Fe, Zn, Mn, I) at three

inclusion levels 1%, 1.5% and 2% respectively. A mechanical pelletiser was used to create sinking pellets (2mm diameter) after all materials had been well combined. The pellets were kept in an airtight container in a cool, dry location until they were needed again after being dried overnight at 40 degrees Celsius in a hot air oven. For 120 days (November 2022- March 2023), fish were fed at a rate of initially 5% and subsequently reduced to 3% of their body weight. Proximate analysis of the test diets (Table 2) and flesh quality were analysed, according to standard procedures (AOAC, 2012).

Blood and serum collection

A 1.0 ml sterile disposable insulin syringe (30G) was used to draw blood from a random sample of five fish from each replication after the fish had been anaesthetised with 30–50 mg L^{-1} of clove oil (1 part clove oil and 9 parts 94% ethanol) on day 120 (Hajek *et al*., 2006). Blood was extracted without the use of heparin, transferred to a 2.0 ml Eppendorf tube, and then chilled in an inclined position for the entire night in order to extract serum. The blood clots were separated from the top by a straw-colored supernatant and centrifuged for 10 minutes at 40^0 C and 2000 rpm. The supernatant was gathered, transferred to a new Eppendorf tube, and kept at -20^0 C for further biochemical analysis in accordance with standard procedure.

Hematological and biochemical parameters

Haemoglobin (Hb), Haematocrit (Ht) or Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), and Total Leukocyte Count (TLC) were measured in the blood (heparinised 150 IU ml^{-1}) drawn from each group. Ht (%) was estimated by micro-capillary method. In the micro capillary method, filled and sealed capillaries are centrifuged at 10,000 rpm for 8 minutes and subsequently final observations are taken from micro- capillary scale and the results were expressed in %.

TEC was measured using the haemocytometer which has neubaur grid on which cell counting areas are marked for the estimation and the technique is popularly known as haemocytometry. It consists of an accurate dilution of measured quantity of blood with a fluid which is isotonic with the blood and prevents coagulation. The blood was drawn into the RBC pipette up to 0.5 mark, followed by sucking of RBC diluting fluid up to 101 mark. This gives a dilution of 1: 200 (Blood: RBC diluting fluid). The solution is mixed by rotating gently and allowed to settle for 2 to 3 minutes. The counting chamber and cover glass were properly cleaned and the cover glass was placed over the ruled area. The solution was mixed gently again and the stem full of solution was expelled and a drop of fluid was allowed to flow under the cover slip by holding the pipette at an angle of 45^o. It was allowed to settle for 2 to 3 minutes, erythrocytes without air bubble under the coverslip were counted. The ruled counting area was focused under the microscope and the number of RBC's were counted in fine small squares of the counting area under high power lenses and number of RBC/mm^{-2} were calculated by using the following formula:

$$\text{Total TEC} = \frac{\text{No. of Cells counted} \times \text{dilution factor (1:200)} \times \text{depth factor (0.1 mm)}}{\text{Total No. of small squares (5)}} \quad (\text{x } 10^6 \text{ mm}^{-3})$$

The acid haematin method was used to measure the amount of haemoglobin (Hb) (Sahli, 1962). The following formulae (Haney *et al*., 1992) were used to compute Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Cell Haemoglobin Concentration (MCHC).

MCV (μm^3)=Ht/RBC x 10, MCH (g%)=Hb/RBCx10

MCHC (g%) = Hb/Ht x 100

Statistical analysis

SPSS software, version 16, was used to do the statistical analysis of the obtained data. One-way analysis of variance (ANOVA) was used to examine the differences between the parameters, and Duncan's multiple comparisons were then used to identify any significant differences. The significance threshold was set at $p<0.05$, and the results were displayed as mean ± SEM.

RESULTS AND DISCUSSION

Physico-chemical parameters

Water temperature, pH, dissolved oxygen, total alkalinity, total hardness, and ammonia do not significantly alter across treatments during the course of the culture period. These fell within the

Table 1: Detail composition (%) of experimental diets*

Ingredients	T1	T2	T3	T4	T5
De-oiled Rice bran	49	49.5	49.25	49	48.75
Mustard oil cake	49	49.5	49.25	49	48.75
Fish Specific Mineral Mixture	0	0	1	1.5	2
Conventional Mineral Mixture	1.5	0	0	0	0
Salt	0.5	0.5	0.5	0.5	0.5

*2% Carboxy methyl cellulose was added as binder above other ingredients

Table 2: Proximate composition of feed ingredients and experimental diets (on % dry matter basis)

Ingredients/ Experimental Diets	Crude Protein	Ether Extract	Crude Fiber	Ash	NFE
De-oiled rice bran	13.83	1.27	15.69	11.32	57.89
Mustard oil cake	39.94	1.97	11.12	7.23	39.74
		Experimental diets			
T1	26.29	3.18	18.99	9.01	42.53
T2	27.56	3.36	19.36	6.88	42.84
T3	27.31	3.19	19.58	8.07	41.85
T4	26.34	3.19	19.46	9.18	41.83
T5	26.23	3.95	19.27	9.42	41.13

Table 3: Growth performance and survival (%) at the end of the experiment*

Parameters	Treatments (with different levels of mineral mix)				
	T1	T2	T3	T4	T5
IW (g)	$15.16^{a}\pm0.19$	$15.54^{a}\pm0.20$	$15.35^{a}\pm0.17$	$15.09^{a}\pm0.43$	$15.08^{a}\pm0.05$
FW (g)	$33.76^{c}\pm0.20$	$31.22^{e}\pm0.24$	$32.53^{d}\pm0.40$	$40.18^{a}\pm0.59$	$36.80^{b}\pm0.20$
WG (%)	$119.96^{c}\pm1.23$	$107.23^{d}\pm6.90$	$114.62^{cd}\pm3.06$	$158.51^{a}\pm2.45$	$143.96^{b}\pm1.09$
SGR (%)	$0.65^{c}\pm0.00$	$0.60^{d}\pm0.02$	$0.63^{cd}\pm0.01$	$0.79^{a}\pm0.00$	$0.74^{b}\pm0.00$
PER	$1.29^{b}\pm0.00$	$1.23^{b}\pm0.02$	$1.27^{b}\pm0.04$	$1.46^{a}\pm0.01$	$1.39^{a}\pm0.00$
FCR	$1.28^{a}\pm0.00$	$1.27^{a}\pm0.01$	$1.26^{a}\pm0.00$	$1.18^{c}\pm0.00$	$1.22^{b}\pm0.00$
Survival (%)	96.66±3.33	93.33±3.33	96.66±3.33	96.66±3.33	96.66±3.33

* Significant differences ($p<0.05$) exist between values (Mean ± S.E.) with various alphabetical superscripts in a row. IW (g): Initial weight, FW (g): Final weight, WG (%): Weight gain %, SGR (%): Specific growth rate, PER: Protein efficiency ratio, FCR: Feed conversion ratio

range that Bhatnagar and Devi (2013) recommended for pond aquaculture in general.

Growth performance and nutrient utilization

Table 3 showed the effects of a fish-specific mineral mixture on the survival, growth performance, and nutritional utilisation of *Cyprinus carpio* fingerling. The growth performance of *C. carpio* fingerlings, including FW (g), WG (%), SGR (%), and FCR, has been significantly ($p<0.05$) increased by formulated mineral supplementation when compared to the non-supplemented group. The T4 group exhibited considerably higher ($p<0.05$) FW (g), WG (%), and SGR (%) than the T1 and T2 treatment groups. Whereas T5 and T3 exhibited comparatively reduced values respectively. Additionally, the T4 group's FCR and PER were much better ($p<0.05$) than those of the T1 and T2 groups, which included conventional and no mineral mixture, respectively. Growth performance started improving with increasing concentration of the formulated mineral mix and observed best @ 1.5% of feed (T4), which is in agreement with the earlier studies (Datta and Kaviraj, 2003; Pandey and Satoh, 2013).

Nutritional composition of the fish

Fish flesh quality (wet weight basis) was assessed at the end of the experiment in terms of total proteins, total lipids, total carbohydrates, ash, and moisture content. The corresponding values are shown in Table 4. At the time of stocking, fish had a 12.31% flesh protein content. Comparing the formulated

Table 4: Initial and final proximate composition of common carp (wet weight basis) in different treatments at the end of the experiment*

Parameters	Initial	Treatments (with different levels of mineral mix)				
		T1	T2	T3	T4	T5
Total Protein	12.31±0.07	13.68^{b}±0.37	12.38^{c}±0.46	12.68^{c}±0.38	15.11^{a}±0.03	14.53^{b}±0.65
Total Lipid	1.51±0.02	1.75^{b}±0.06	1.66^{c}±0.08	1.80^{b}±0.12	2.10^{a}±0.31	1.94^{ab}±0.06
Total Carbohydrate	2.26±0.01	2.30^{a}±0.29	2.23^{a}±0.14	2.21^{a}±0.18	2.25^{a}±0.27	2.30^{a}±0.32
Ash	1.91±0.02	2.14^{a}±0.15	1.81^{c}±0.78	2.43^{c}±0.19	2.07^{b}±0.52	2.07^{b}±0.07
Moisture	82.01±0.05	80.13^{c}±0.40	81.92^{a}±0.10	80.88^{b}±0.25	78.47^{d}±0.80	79.16^{b}±0.21

*Significant differences (P<0.05) exist between values (Mean ± S.E.M.) with various alphabetical superscripts in a row

Table 5: Haematological parameters of common carp in different treatments at the end of the experiment*

Parameters	Treatments (with different levels of mineral mix)				
	T1	T2	T3	T4	T5
Hb (g%)	7.80^{c}±0.09	5.86^{e}±0.09	6.79^{d}±0.09	9.80^{a}±0.09	8.56^{b}±0.11
PCV/Hct (%)	38.01^{c}±0.08	32.58^{d}±0.19	38.08^{c}±0.46	43.26^{a}±0.76	40.72^{b}±0.49
TEC (x10^{6} mm^{3-1})	1.70^{c}±0.01	1.06^{e}±0.01	1.42^{d} ± 0.04	2.06^{a}±0.04	1.92^{b}±0.036
TLC (x10^{3} mm^{3-1})	4.96^{c}±0.05	2.92^{d}±0.08	2.92^{d}±0.09	7.04^{a}±0.09	5.92^{b}±0.05
MCV (μm^{3})	196.79^{c}±0.82	247.71^{a}±1.61	210.46^{b}±1.04	178.11^{e}±0.77	183.42^{d}±0.53
MCH (g%)	31.58^{b}±0.71	29.09^{c}±0.48	29.98^{c}±0.13	33.16^{a}±0.57	32.83^{ab}±0.22
MCHC (g%)	21.28^{a}±0.072	19.32^{b}±0.085	21.44^{a} ± 0.58	21.69^{a}±0.064	21.67^{a}±0.041

* Significant differences (P<0.05) exist between values (Mean ± S.E.) with various alphabetical superscripts in a row. Haemoglobin (Hb), Hematocrit (Ht) or Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Cell Hemoglobin Concentration (MCHC)

fish-specific mineral mixture to the positive (T1), negative control (T2), and initial fish, the results showed a significant (p<0.05) improvement in flesh quality in terms of total protein content (13.68, 12.38, 12.68, 15.11, 14.53% in T1 to T5 respectively). Similarly, at the time of stocking, the total lipid content was 1.51%, which significantly (p<0.05) improved with increasing dosages of the mineral mix (1.75, 1.66, 1.80, 2.10, 1.94% in T1 to T5 respectively). The best results were obtained at 1.5% of feed, with a decrease in ash and moisture content when compared to when no minerals were present in the feed. Overall results revealed that formulated mineral supplementation in the feed improved the flesh quality of common carp and observed best @1.5% in feed which is in agreement with the earlier studies (Lall and Kaushik, 2021; Musharraf and Khan 2022).

Haematological parameters

At the conclusion of the experiment, the fish's haematological parameters—such as haemoglobin (Hb), haematocrit (Ht) or packed cell volume (PCV), total erythrocyte count (TEC), total leucocyte count (TLC), MCV, MCH, and MCHC—were examined and computed (Table 5). The levels of Hb, PCV, TEC, TLC, MCH, and MCHC rose in the mineral-supplemented group. Additionally, MCV improved as supplementation increased from 0 to 1.5% of feed, with a significant (p<0.05) improvement in T4 when compared to the negative control group, which had no additional mineral content (Table V). The best results were obtained at 1.5% of feed.

The current study supports previous research (Datta and Kaviraj, 2003) that found minerals are often added to carp feed at a rate of 1% to 2% throughout the formulation process. Musharraf and Khan (2022) investigated the mineral needs of Indian major carps in similar research. However, according to Storebakken *et al*. (2000), the amount of minerals provided by the animal and plant feed ingredients employed in the artificial feed formulation is substantial and frequently exceeds the projected demand. Salmonids and certain warm-water fishes were the subjects of early mineral nutrition research, which used semi-purified diets and highly

bioavailable trace element supplementation. Pandey and Satoh (2013) worked on common carp and concluded that 2% of monocalcium phosphorous satisfies phosphorous requirements of the fish. Whereas, maximum weight gain was obtained at 0.7% phosphorous level regardless of dietary calcium level in case of carp (Ogino and Takeda, 1976). Na and K, the main internal and external cations, contribute to ionic balance; Ca, P, and Mg are important components for the hard structure of the organism, such as scales and bones (Lall, 2002). Zinc in water up to 0.10mg/l resulted in increased growth of fish in FRP tanks, beyond this it has detrimental effect on growth of carp fingerling (Mohanty *et al.,* 2009). Exposure to sub-lethal concentration of copper sulphate results in decrease in haemoglobin and other haematological parameters (Singh *et al.*, 2008). Zooplankton considered as major source of cobalt and selective preference of zooplankton shows better growth in common carp compared to *Heteropneustes fossilis* fed with 2% mineral diet (Mukherjee and Kaviraj, 2009). Fish feed with 30% protein, 7.9% lipid, 2% mineral-vitamin mixture are seen effective for growth of Chinese carp in mid-hilly regions of Uttarakhand (Mehta *et al.,* 2020). Higher growth rate observed in grass carp and common carp fed with Agrimin compared to Fishmin, may be due to presence of higher mineral content and presence of methionine and L-lyine, Mono-HCl in its composition (Sudhakar *et al.,* 2015). Fish mineral absorption and utilisation can be impacted by anti-nutritional factors (ANFs) as phytic acid, gossypol, oxalates, glucosinolates, saponin, lectin, tanin, and even non-starch polysaccharides (Francis *et al*., 2001). For young fish, the most well-known mineral needs (Ca, P, Mg, Cu, Zn, Mn, and Se) were identified. Recommendation allowances for new fish species have been established using the NRC (2011) requirement values for specific minerals for around ten fish species as a guide. The food intake or mineral status before to the experimental period, as well as the impact of the previous diet on body reserves at the start of the study, were not given much thought in many short-term experiments. Depending on the temperature of the water, certain minerals require more time to stabilise after a change in their dietary intake. New trace element supplements are also available, but they need to be properly evaluated.

CONCLUSION

In conclusion, when compared to the non-supplemented group (T2), dietary supplementation of formulated fish-specific mineral mixtures improved the growth, nutrient composition and health status of *Cyprinus carpio* fingerlings and resulted best at 1.5% mineral mixture in fish fed. The result obtained in this study will be helpful for various stakeholders including fish farmers, entrepreneurs and feed industry.

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