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Potential of *Moringa oleifera* Leaf Meal as Substitute of Fishmeal on Performance of *Catla catla* Fingerlings Reared in Earthen Ponds

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MF conducted the study and wrote the manuscript. SMH administered and supervised the project, and acquired funds. MH, ZY, MA and MZHA curated the daata and helped in writing, editing and reviewing the manuscript.

Key words
Moringa leaf meal, *Catla catla*, Growth performance, Body mineralization

ABSTRACT

This study was carried out in a semi-intensive rearing system in order to assess the impact of *Moringa oleifera* leaf meal (MOLM) based diets on the overall performance of *Catla catla* fingerlings cultured in 6 earthen ponds. In order to formulate experimental diets, MOLM was used as test ingredient. MOLM was used to replace fish meal (FM) in the diet at varying levels of 0%, 10%, 15%, 20%, 25%, and 30%, respectively. There were five experimental diets and one control diet used during the trial. Fingerlings were placed in a cemented pond for fifteen days under laboratory conditions for acclimatization. There were 15 fingerlings put into each of the six earthen ponds, for a total of 90 fingerlings. Analyses showed that fish fed with 10% MOLM-based diet had the highest growth performance when compared with other experimental and control groups. Best results for carcass composition, hematological analysis, and body mineralization were noted in fingerlings given a diet containing a 10% substitution of FM with MOLM. In the present investigation, it was determined that replacing 10% of FM in the diet with MOLM improved the growth, body composition, hematological parameters, and body mineralization of *C. catla* fingerlings.

INTRODUCTION

All living organisms, including fish, need nutritious food to survive, reproduce, and maintain their bodies (Adebayo *et al*., 2020). The aquaculture industry is growing at a rate of around 5.8% each year as a result of the tremendous growth and development occurring throughout the sector (FAO, 2020). It is the fastest developing industry and provides 50% of entire worldwide food fish utilization (FAO, 2010). There has been a rise in the demand for food and the need of a healthy diet in developing nations as a consequence of their rapid population expansion (Abdulkadir *et al*., 2016). Fish and fish by products play an important part in meeting the needs of the human population in terms of food security as well as the nutritional requirements of both emerging and developed nations (FAO, 2014). It accounts for 17% of animal protein and 7% of entire protein consumed worldwide (FAO, 2020).

Fish meal (FM) is a significant contributor of protein to the diets of farmed fish (Dawood *et al*., 2015). The aquaculture industry utilizes 3.06 mt of it since it is a significant source of protein (FAO, 2008). In aquaculture, fish feed contributes around 40-60 % of production costs (Nasr *et al*., 2021). Furthermore, from last 30 years, FM cost have also increased in right terms. The rapid rise in the price of fish oil and FM may possess a negative impact on commercial aqua-operations (Naylor *et al*., 2000). Unpredictable availability, rising demand, and a greater value for fishmeal lead it necessary to look for new protein sources (Hussain *et al*., 2015a).

Plant-based protein sources have largely replaced fish meal as fish feed (Elumalai *et al*., 2021) because they are more readily accessible, less harmful to the environment, more sustainable, and cheaper (Hardy, 2010). One excellent protein source is the *Moringa oleifera* (miracle tree), which can be incorporated into aquaculture diets. This plant is often found in tropical and subtropical regions

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and is also known as drumstick tree. It is used in variety of applications, both in industry and medicine (Saini *et al*., 2016; Falowo *et al*., 2018). The most commonly used part of this plant is its leaves that are important source of polyphenols, flavonoids, alkaloids, saponins, tannins, glucosinolates, carotenoids and vitamins (Leone *et al*., 2015). For the purpose of improving growth and immunological function, *M. oleifera* leaf meal (MOLM) has been used in aquaculture diets as a research subject (Puycha *et al*., 2017). It has been found that moringa leaves have high ratio of vitamins A and C, iron, potassium, calcium and proteins (25 to 32%) than other products of food like banana, orange, yoghurt, carrot and milk (Soliva *et al*., 2005; Gopalakrishnan *et al*., 2016). In aquaculture technology, moringa has become possible substitute plant protein source, moreover, its leaves contain important amino acids, especially tryptophan, lysine, methionine and cysteine (Sherif *et al*., 2022).

Catla catla feeds on surface and called as surface feeder that is commonly used in Pakistan's polyculture technique and is grown along with other varieties (Aslam *et al*., 2016). Its output increased in the first decade of the first-twenty century, reaching roughly 2.8 mt per year in 2012 (FAO, 2010). As a result, the current study aimed to examine the efficacy of MOLM to serve as an alternative source of protein in formulated diets of fingerlings as well as to evaluate its effects on growth performance, body composition, hematology and mineral status of *C. catla* fingerlings when cultured in earthen ponds.

MATERIALS AND METHODS

Study area and pond preparation

Six-month feeding trial was done between February and August 2021 in six earthen ponds using a semi-intensive rearing method at the Fisheries Research Farms, Department of Zoology, University of Agriculture Faisalabad, Pakistan. Furthermore, analysis of feed ingredients and fish body were carried out at Fish Nutrition Laboratory, Government College University, Faisalabad, Pakistan. Each pond had a rectangular form. The ponds were irrigated by a nearby groundwater tube well. Every pond had an entrance and an outlet for water supply. Prior to the start of the experimental trial, ponds were rebuilt, aquatic flora, fauna and fish were removed. Before fertilization, agricultural lime ($CaCO_3$) was provided to all ponds at a rate of 250 kg/ha to maintain pH of the ponds and filled with water for 7 days. After that, ponds were fertilized with both organic and inorganic fertilizers to keep planktonic biomass for fingerlings. Both organic and inorganic fertilizers were uniformly applied to each pond on fortnightly basis for a period of six months.

Fish and experimental conditions

Fingerlings were purchased from Government Fish Seed Hatchery, Satiana Road, Faisalabad and transported to Department of Zoology, Government College University, Faisalabad. Fingerlings were placed in a cemented pond for 15 days under laboratory conditions for acclimatization. At 10:00 A.M. the fingerlings were fed to satiation with the feed. An air pump provided continuous air to the fingerlings via capillary system. After acclimatization, the fish were divided into groups and then transported to the Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. NaCl (5g/L) was applied to fingerlings before to the start of feeding experiment to kill any ectoparasites present and prevent any fungal infections (Rowland and Ingram, 1991). In order to maintain the level of dissolved oxygen (DO), air was provided to each pond by changing water. Physical and chemical parameters were monitored on regular basis, including temperature, pH, DO, and carbon dioxide. Prior to the start of the experiment, wet weight, fork length and total length of all groups of fingerlings were measured.

Experimental design

In order to formulate an experimental diet, MOLM was used as test ingredient. One group was kept as control for this experiment and received only organic and inorganic fertilizers. However, the other groups were treated with different levels of MOLM based diets along with fertilizers. During the trial, total 90 fingerlings were distributed among 6 earthen ponds. Each pond received fifteen fingerlings, which were nourished at a ratio of 3% of their biomass each day. The six-month feeding study was conducted.

Moringa oleifera *leaf processing and other feed ingredients*

The feed ingredients were transported from Multan in southern Punjab. The crushed leaves were first treated by soaking in tap water at room temperature for three days. In order to reduce anti-nutritional substances such as saponins, the mixture was stirred daily for one hour while soaking (Tacon and Jackson, 1985; Wee and Wang, 1987). The mixture was soaked and then the water was removed by squeezing it through a cloth. The grinded materials for the experimental diet were tested for their chemical make-up before they were blended (AOAC, 2005). Chrome oxide served as an inert marker in the experimental diets. Six test diets were prepared using MOLM to replace fishmeal at levels of 0%, 10%, 15%, 20%, 25%, and 30%.

Table I. Composition of ingredients (%) in test diets.

Ingredients	Test diet-I (control)	Test diet-II	Test diet-III	Test diet-IV	Test diet-V	Test diet-VI
Replacement level	0 %	10 %	15 %	20 %	25 %	30 %
MOLM*	0	10	15	20	25	30
Fish meal	50	40	35	30	25	20
Wheat flour**	17	17	17	17	17	17
Corn gluten	20	20	20	20	20	20
Fish oil	9	9	9	9	9	9
Vitamin premix	1	1	1	1	1	1
Mineral premix	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1

MOLM*, Moringa oleifera leaf meal.

Formulation and processing of experimental diets

A 0.5 mm sieve was used to finely grind the feed materials. After 5 min of mixing all of the contents in a mixer, fish oil was gradually added. After adding 10–15% water to the feed ingredients, the mixture was incorporated into the mixer to generate dough with an appropriate texture, which was then processed through an experimental extruder to produce pellets that floated (Lovell, 1989).

Proximate analysis

The whole body and experimental diet samples were individually homogenized using a motor and pestle prior to being analyzed utilizing standard procedure. Crude protein (N×6.25) was assessed by micro Kjeldahl apparatus; moisture by oven drying at 105°C for 12 h; crude fat by petroleum ether extraction procedure using Soxtec HT2 1045 system and ash was calculated by electric furnace ignition at 650°C for 12 h (Eyela-TMF 3100). Mineral analysis of fingerlings was determined by atomic absorption spectrophotometer (AOAC, 2005).

Growth study

After the completion of trial, all fish were weighed to assess growth.

Hematological parameters

Blood was obtained from the caudal vein of *C. catla* fingerlings using a syringe (heparinized) and delivered to the Department of Zoology, UAF, for hematological investigation. The micro-hematocrit approach was applied to assess hematocrit using capillary tubes (Brown, 1988). To count RBCs (red blood cells) and WBCs (white blood cells), a hemocytometer with an authorized Neubauer counting chamber was utilized (Blaxhall and Daisley, 1973). As indicated by Wedemeyer and Yastuk (1977), Hb (hemoglobin) concentration was determined. Standard formulae were used to compute MCHC (mean corpuscular hemoglobin concentration), MCH (mean corpuscular hemoglobin), and MCV (mean corpuscular volume) (mean cell volume).

MCHC = Hb/PCV x 100
MCV = PCV/RBC x 10
MCH = Hb/RBC x 10

Data analysis

The data on different parameters of growth, body composition, hematological parameters and body mineralization were statistically analyzed using a microcomputer, as described by Steel *et al.* (1996). One-way analysis of variance (ANOVA) was used to analyze the data (Steel *et al.*, 1996). Tukey's Honesty Significant Difference Test was used to compare the differences among means (Snedecor and Cochran, 1991). For statistical analysis, the Co-Stat computer software (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

RESULTS

Growth performance

Table II describes the growth parameters of *C. catla* fed with MOLM based diet, in terms of final weight and weight gain. Maximum value of final weight (677.42 g) was obtained when fingerlings given 10% MOLM based diet while minimum value of final weight (438.39 g) was recorded at group fed 30% inclusion of MOLM based diet. Highest weight gain (662.09 g) of fingerlings was observed when fingerlings fed at 10% of MOLM based diet while lowest value of weight gain (432.15 g) was recorded when fingerlings fed at 30% inclusion of MOLM based diet. It was indicated that, there was an increasing tendency in final weight and weight gain when fingerlings fed 10% MOLM based diets. Moreover, significant difference was observed in the final weight and weight gain when compared to control diet and all other test diets. Current study revealed that 10% inclusion of MOLM in the diets improved the growth performance of fingerlings.

Body composition

Table III provides an explanation of the body composition of *C. catla* fingerlings treated for 90 days on various MOLM based diets. The best value of CP (20.81%) and poor value of CF (2.04%) in the fingerlings body were observed at group II having 10% substitute level of MOLM based diet. These values were statistically significant (p<0.05) from fingerlings treated 0%, 20%, 25%, and 30% replacing level MOLM based diets as represented in table. Lowest value of CP (16.14%) and greatest value of CF (6.56±0.14%) were found in the group VI that fed on the 30% replacement of MOLM based diet. Highest value of ash (3.10%) was found in the group II having replacement level of 10% MOLM based diet. While lowest value of ash (2.41%) content was observed in the group VI having substitute level of 30% MOLM based diet. It was also noticed that protein and ash contents were highest in group II having 10% substitution level of MOLM based diet as compared to other diets such as 0%, 20%, 25% and 30% replacement levels of MOLM based diets. The greatest value of moisture (74.88%) was found in group VI fed 30% replacement level of MOLM based diet. While lowest value of moisture (74.03%) was found in group II fed 10% replacement level of MOLM based diet. It was observed that substitution of 10% MOLM based diet improved the body composition of fingerlings, while increased level was non-significant.

Hematological parameters

Table IV is representing the results of hematological parameters fed fingerlings with inclusion of MOLM based diet. Maximum value of RBCs (2.80×10^6 mm^{-3}) was showed by fingerlings fed 10% MOLM based diet while lowest value of RBCs (2.32×10^6 mm^{-3}) was recorded in the fingerlings when fed with 30% MOLM based diet. Maximum WBCs (7.82×10^3 mm^{-3}) were recorded in the group II having 10% replacement of MOLM based diet while lowest value of WBCs (7.17×10^3 mm^{-3}) was found in the group VI having 30% MOLM based diet. The best values of RBCs and WBCs fed 10% MOLM based diets were statistically significant from control diet and all other diets. Highest value of PLT (64.79) was observed in the

Table II. Analysis of final weight and weight gain of *C. catla* fingerlings fed with MOLM based diets.

Experimental diets	MOLM replacement level	Initial weight (g)	Final weight (g)	Weight gain (g)
Group I (Control)	0%	15.34±0.03	514.43±0.24^d	499.08±0.25^d
Group II	10%	15.33±0.06	677.42±0.19^a	662.09±0.24^a
Group III	15%	15.25±0.03	596.40±0.23^b	581.15±0.20^b
Group IV	20%	15.34±0.03	553.63±0.16^c	538.29±0.14^c
Group V	25%	15.22±0.03	476.42±0.03^e	461.19±0.05^e
Group VI	30%	15.24±0.05	438.39±0.14^f	432.15±0.09^f

Means within rows having different superscripts are significantly different at (p< 0.05).

Table III. Body composition of *C. catla* fingerlings fed on various replacement levels of MOLM based diets.

Experimental diets	MOLM replacement level	CP (%)	CF (%)	Ash (%)	Moisture (%)
Group I (Control)	0%	19.30±0.22^c	3.38±0.15^d	2.96±0.01^b	74.35±0.08^{bcd}
Group II	10%	20.81±0.13^a	2.04±0.09^f	3.10±0.01^a	74.03±0.03^d
Group III	15%	18.40±0.13^d	4.34±0.13^c	2.83±0.04^c	74.41±0.05^{bc}
Group IV	20%	20.01±0.10^b	2.81±0.30^e	2.98±0.06^{bd}	74.18±0.18^{cd}
Group V	25%	17.33±0.22^e	5.38±0.18^b	2.69±0.02^d	74.59±0.08^{ab}
Group VI	30%	16.14±0.09^f	6.56±0.14^a	2.41±0.04^e	74.88±0.20^a

Means within rows having different superscripts are significantly different at (p< 0.05). Data are means of three replicates.
CP, crude protein; CF, crude fat.

Table IV. Hematological parameters *C. catla* fingerlings fed on various replacement levels of MOLM based diets.

Experimental diets	MOLM (%)	RBCs (10^{-6} mm^{-3})	WBCs (10^{-6} mm^{-3})	PLT	Hb (g/100ml)	MCHC (%)	PCV (%)	MCV (fl)	MCH (pg)
Group I (Control)	0	2.42±0.01^{c}	7.36±0.07^{c}	58.45±1.00^{d}	7.53±0.05^{d}	28.31±0.09^{d}	24.21±0.09^{c}	107.68±4.07^{d}	38.13±0.57^{d}
Group II	10	2.80±0.08^{a}	7.82±0.08^{a}	64.79±0.17^{a}	8.76±0.05^{a}	34.81±0.08^{a}	26.63±0.02^{a}	178.27±2.09^{a}	53.92±0.95^{a}
Group III	15	2.65±0.02^{b}	7.69±0.02^{a}	62.56±0.11^{b}	8.32±0.03^{b}	33.02±0.68^{bb}	25.35±0.06^{b}	153.79±1.96^{b}	48.76±1.04^{b}
Group IV	20	2.55±0.02^{b}	7.52±0.02^{b}	60.73±0.13^{c}	7.88±0.12^{c}	30.45±0.13^{c}	23.41±0.05^{d}	125.61±2.70^{c}	44.57±0.90^{c}
Group V	25	2.38±0.00^{c}	7.27±0.03^{cd}	57.31±0.07^{d}	7.11±0.11^{e}	24.50±0.61^{e}	22.14±0.03^{e}	94.85±2.98^{e}	33.26±1.05^{e}
Group VI	30	2.32±0.03^{c}	7.17±0.01^{d}	53.18±1.04^{e}	6.95±0.07^{e}	21.65±0.54^{f}	21.91±0.06^{f}	86.93±1.80^{f}	29.09±0.10^{f}

RBC, red blood cell; WBC, white blood cell; PLT, platelet; Hb, hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin. Means within rows having different superscripts are significantly different (p<0.05). Data are the mean of three replicate.

Table V. Whole body mineralization *C. catla* fingerlings fed on various replacement levels of MOLM based diets.

Experimental diets	MOLM (%)	Zn (µg/g)	P (%)	Ca (%)	Na (mg/g)	Mg (%)	Fe (µg/g)	Mn (µg/g)	K (%)	Cu (µg/g)	Se (mg/g)
Group I (Control)	0	3.43±0.03^{d}	1.05±0.01^{c}	0.87±0.02^{ab}	5.11±0.02^{d}	2.69±0.04^{d}	47.55±0.19^{d}	8.21±0.02^{d}	7.77±0.01^{d}	3.33±0.02^{d}	0.36±0.01^{b}
Group II	10	4.13±0.02^{a}	1.24±0.03^{a}	1.00±0.03^{a}	5.83±0.04^{a}	3.27±0.01^{a}	61.44±0.10^{a}	12.15±0.01^{a}	8.68±0.02^{a}	4.08±0.02^{a}	0.32±0.02^{b}
Group III	15	3.98±0.01^{b}	1.16±0.01^{b}	0.97±0.02^{cd}	5.69±0.02^{b}	3.05±0.02^{b}	54.63±0.06^{b}	11.09±0.01^{b}	8.19±0.01^{b}	3.92±0.02^{b}	0.24±0.01^{c}
Group IV	20	3.77±0.04^{c}	1.11±0.02^{bc}	0.92±0.01^{bc}	5.41±0.02^{c}	2.87±0.02^{c}	51.64±0.25^{c}	9.33±0.02^{c}	7.96±0.02^{c}	3.62±0.04^{c}	0.23±0.01^{c}
Group V	25	3.01±0.02^{e}	0.97±0.03^{d}	0.81±0.01^{de}	3.98±0.01^{e}	2.37±0.02^{e}	37.43±0.13^{e}	7.79±0.02^{e}	6.91±0.02^{e}	2.87±0.01^{e}	0.38±0.02^{b}
Group VI	30	2.23±0.04^{f}	0.89±0.02^{e}	0.76±0.02^{e}	3.16±0.01^{f}	1.97±0.02^{f}	33.59±0.06^{f}	7.49±0.02^{f}	6.09±0.05^{f}	2.16±0.01^{f}	0.49±0.03^{a}

Means within rows having different superscripts are significantly different at (p< 0.05). Data are means of three replicates.

group II having 10% inclusion of MOLM based diet while lowest value of PLT (53.18) was recorded in the group VI having 30% MOLM based diet. Maximum value of Hb (8.76 g/100ml) was found in the fingerlings when fed with 10% MOLM based diet while poorest value of Hb (6.95 g/100ml) was indicated by fingerlings fed 30% MOLM based diet. Best values of PCV, MCHC, MCH and MCV (26.63%, 34.81%, 53.92 pg and 178.27fl, respectively) were indicated in the fingerlings fed 10% inclusion of MOLM based diet while minimum values of PCV, MCHC, MCH and MCV (21.91%, 21.65%, 29.09 pg and 86.93 fl, respectively) were recorded in the fingerlings having 30% MOLM based diets. These findings of current study, suggested that inclusion of 10% MOLM in the diet improved hematological indices of fingerlings.

Body mineralization

The body mineralization of *C. catla* treated on various levels of MOLM-based diet is indicated in Table V. Body mineral contents of the fingerlings revealed that there were significant differences (p<0.05) between them. Best values of Zn, P, Ca, Na, Mg, Fe, Mn, K and Cu (4.13 µg/g, 1.24%, 1.00%, 5.83 mg/g, 3.27%, 61.44 µg/g, 12.15 µg/g, 8.68% and 4.08 µg/g) were deposited in the fingerlings body recorded at group II having 10% replacement of MOLM based diet and these values were significantly different (p<0.05) from the fingerlings fed on other experimental diets. While minimum values of Zn, P, Ca, Na, Mg, Fe, Mn, K and Cu (2.23 µg/g, 0.89%, 0.76%, 3.16 mg/g, 1.97%, 33.59 µg/g, 7.49 µg/g, 6.09 % and 2.16 µg/g) were observed in the fingerlings body fed 30% replacement of MOLM based diet. Maximum value of Se (0.49 mg/g) was found in the fingerlings body at group VI having 30% inclusion of MOLM based diet while lowest value (0.23 mg/g) of Se was indicated in the group IV.

DISCUSSION

Our research aimed to examine the effects of six various levels of MOLM based diet on growth performance, body composition, hematological indices and body mineralization in *C. catla* fingerlings. Current study revealed that feeding *C. catla* fingerlings with 10% MOLM based diet in the replacement of fishmeal improved the overall growth performance. The growth rate of Nile tilapia improved by MOLM based diet, according to a recent

Egyptian study (Elabd *et al*., 2019). Findings of this study agreed with results of Doctolero and Bartolome (2019) who used MOLM in the diets of *Oreochromis niloticus* and indicated that up to 20% replacement of MOLM improved the growth performance of fish. In the same way, findings of this study are also similar with results of Elabd *et al*. (2019) who used diet based on MOLM for Nile tilapia and found that growth of fish was significantly increased. Moreover, he concluded that supplementing Nile tilapia with moringa based diets had beneficial effects as a growth stimulant. Results of Hussain *et al*. (2018) also showed that 10-20% inclusion of MOLM in the diet of *Labeo rohita* significantly increased the weight gain of fish while further increase in the concentration of MOLM had not satisfactory effect on growth performance of fish. Current study results were also in line with the findings of Idowu *et al*. (2017) who revealed that 15% inclusion of MOLM in the diets of *Clarias gariepinus* post-fingerlings significantly enhanced the weight gain of fish while 25% inclusion of MOLM in the diet, lower the mean weight gain of fish. Tabassum (2017) also recorded that 10% inclusion of MOLM in diet of Mozambique tilapia improved the growth performance of fingerlings. MOLM was proved as a fish meal alternative in the diet of *C. gariepinus* up to 10% without impairing growth, according to Ezekiel *et al*. (2016). Our results were dissimilar with outcomes of Khetran *et al*. (2018) who observed that addition of 30% moringa meals in diets of *L. rohita* can boost the growth. The higher growth rates could be linked to the high antioxidant contents of moringa leaves, such as vitamin C and carotene, which are favorable for fish health (Vergara-Jimenez *et al*., 2017). This research also concluded that 30% inclusion of MOLM in the diet didn't show significant results, because the increasing MOLM in the diet contains lots of fiber.

The findings of current study match with those of Arsalan *et al*. (2016) who reported that 10% replacement level had the greatest CP when compared to a control group while 40% incorporation had the lowest CP. It was also shown that adding 10% MOLM to fish feed is a significant protein substitution. Similarly, Ganzon-Naret (2014) studied the carcass composition of Asian sea bass at various inclusion levels of MOLM based diets and indicated maximum CP value at 10% which was statistically significant than other MOLM based diets. Hence, according to current study, an excellent alternate source of protein for fingerlings' diet is 10% replacement level of MOLM.

The growth and health of fish are now reflected by hematological parameters, which are key factors used in aquaculture (Fazio, 2019). Present study findings also similar to Tabassum *et al*. (2023), those who indicated that MOLM increased in the diet of *Cirrhinus mrigala* showed decreased improvement in hematological studies of fish and suggested that 10% replacement of MOLM in the diet had beneficial effects on the hematology of fish. In the same way, Billah *et al*. (2020) also observed that increasing the MOLM in the diet of fish declined the improvement of RBCs and WBCs in *O. niloticus*. The results of Arsalan *et al*. (2016), indicated parallel results with current study, those who showed that 10% inclusion of MOLM had positive effects on the hematology of *L. rohita* but hematological analysis revealed that MOLM inclusion of more than 20% in the diets caused hematological disturbance. The findings of the current investigation were in conflict with those of El-Gawad *et al*. (2020), who noted a significant increase in WBCs when fish fed with 1.5% moringa leaf powder whereas, Hb and RBCs amounts in the *O. niloticus* not significantly improved. It might be due to size of fish, concentration of dose, variety of fish and health condition of fish. According to the findings of current research, 10% MOLM based diet had improved the hematological indices of fish in earthen ponds.

Minerals are naturally occurring inorganic substances that are required for normal functioning of the fish body. In the current study, results indicated that highest values of minerals such as Zn, P, Ca, Na, Mg, Fe, Mn, K and Cu were observed in the body of fingerlings fed 10% inclusion of MOLM based diet while lowest values of minerals as like Ca, Na, P, Mg, Fe, Mn, Zn, K and Cu were found in group VI having 30% replacement of MOLM based diet. Whereas highest value of Se was found in the group VI having 30% addition of MOLM based diet but lowest value of Se was observed in the fingerlings body fed 20% MOSM based diet. Our results, were similar to Shahzad *et al*. (2021), who found that addition of MOLM in diet of common carp had improved the mineral contents. Present research work, revealed that inclusion of 10% MOLM in the diet of fish significantly boosted the mineral contents of *C. catla*. While increasing the concentration of MOLM in the diet of fish lowered the body mineralization of fish.

CONCLUSION

It was deduced that 10% replacement of fish meal with MOLM in the diet causes considerable improvement on overall response of fingerlings while increasing the levels of MOLM in the diet didn't show significant effects. Additionally, it was confirmed that MOLM could replace costly FM by up to 10–20% without negatively affecting the overall response of *C. catla* fingerlings.

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Ethical statement

All applicable international, national, and/orinstitutional guidelines for the care and use of animals were followed.

Statement of conflict of interest

The authors have declared no conflict of interest.

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