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Efficacy of MCP Supplemented Plant Meal-Based Diet on Performance of *Labeo rohita* Fingerlings

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FM executed the feeding trial and composed the manuscript. MMS planned, oversaw, and supplied all requisite materials for the research endeavor. UE contributed to the manuscript preparation process. FY and AK assisted in the composition, review, and refinement of the document. ZH participated in the editing and restructuring of the manuscript.

Key words
Sesame meal, Growth performance, Nutrient digestibility, Immunology

ABSTRACT

A 10-week growth trial was carried out to assess the impact of a plant meal-based diet supplemented with monocalcium phosphate (MCP) on growth performance, nutrient digestibility, and immunology. Six isonitrogenous and isocaloric diets were prepared five of which included MCP supplementation (0.20%, 0.40%, 0.60%, 0.80%, 1%) and one was a control (0%). Replicate groups of each test diet were fed on their particular diets @ 4% of their live wet weight. The highest values for maximal weight gain (19.62 g), final weight (26.61 g), and weight gain percentage (280.93%) were observed in the fish groups fed with diet IV (0.60% supplementation of MCP). Similarly, gross energy (GE), digestibility and crude protein digestibility were found best in the fingerlings that were given test diet IV (0.60% MCP), and fish fed with 0.40% MCP supplemented diet showed the highest crude fat digestibility. The test diet supplemented with 0.60% MCP provides the highest immunological indices WBC ($6.65 \times 10^3 mm^{-3}$) and lymphocytes (26.32%). The findings indicate to achieve improved fish growth, nutrient digestibility, and immunology there is a need for supplementation of MCP up to 0.60% in a plant meal-based diet for rohu fingerlings.

INTRODUCTION

Globally, the world population has dramatically increased to 7.9 billion in 2020, that was 1 billion in 1800 (Eniayejuni, 2020). UN projections predict that total population will reach 11.2 billion by 2100 (McNicoll, 1992). Food insecurity and the strain on natural resources are exacerbated due to population rise (Uniyal *et al*., 2020). With 11% of the world's population already facing hunger and having no access to proper nutrient-rich food (FAO, 2018). Aquaculture becoming the fastest-growing food production will provide about 50% of the world's edible fish consumption by 2030 and should be able to supply 60-70% of the demand (Subasinghe *et al*., 2009). Fish farming currently produces more than other food production industries with an annual production of 76.6 million tons (FAO, 2017). There is about 15-16% contribution of fisheries products in total consumption of animal based sources among 2.9 billion people that are in low-income stage (Ayoola, 2010). About 3.1 billion individuals get their daily animal protein intake from fish and seafood, which makes a substantial contribution to global nutrition (FAO, 2017). Fish is an abundant source of vital AAs, minerals, high-quality protein, Ω 3 fatty acids as well as vitamins with production growing from 19 million metric tons in 1950 to 171 million mt in 2016 (Amescua, 2023). The aquaculture industry's viability depends on delivering high-nutritional and reasonably priced diets (Rao *et al*., 2020). A major source of protein for fish feed is fish meal which is becoming more in demand making its availability difficult and feed formulation expensive (Acar and Turker, 2018). Due to competition and limited fishing resources fish meal has become more expensive making fish feeds unaffordable for many farmers (Hardy, 2010). Consequently, the use of plant-based proteins (such as soybean and maize gluten meal) and animal by products as substitute sources of protein is becoming more frequent in aqua-feeds (FAO, 2011). Despite the difficulties in

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obtaining traditional fish meals, these substitutes aid in maintaining aquaculture productivity (Mallioris *et al*., 2022). Plant proteins are becoming well-known as viable alternatives to fish meal in fish diets due to the high cost and irregular supply of fish meal (Akter *et al*., 2023). Plant protein sources are more widely accessible and usually sell for less money than fish meal (Chakraborty *et al*., 2019). Researchers have made substantial progress toward replacing fish meal with plant proteins which opens up new possibilities for aquaculture in developing nations (Torstenson *et al*., 2008). A cheap and high-protein alternative to fish feed is sesame (*Sesamum indicum*) meal which is a by-product after oil extraction (Hassan *et al*., 2018). Fish can efficiently digest the nutritional components of sesame seeds which are greater in Ω 6 fatty acids making them a good choice for fish diets (Onsaard *et al*., 2010). Plant-based phosphorus is frequently in the form of phytate which fish cannot efficiently utilize plant protein sources in fish diet can result in phosphorus (P) insufficiency (Agrahar *et al*., 2018). Fish diets are supplemented with inorganic phosphorus such as di- or mono-calcium phosphate (DCP or MCP) to address this problem. Fish that are deficient in phosphorus may grow poorly have skeletal malformations, and accumulate lipids because phosphorus is essential for fish growth, bone mineralization, and their metabolism (Costa *et al*., 2018). Aquatic organisms play a crucial role in human nutrition and global aquaculture. However, these ecosystems are increasingly threatened by pollution, including hazardous chemicals that adversely affect aquatic species and their habitats (Selamoglu, 2020). Fish health, essential to these systems, is particularly vulnerable to oxidative stress exacerbated by pollution (Selamoglu, 2021a; Kakoolaki *et al*., 2013). Moreover, optimal nutrition is vital for promoting fish health and ensuring sustainable aquaculture practices, with recent studies exploring plant-based feeds enriched with essential nutrients to counteract environmental toxins (Ates *et al*., 2008; İbrahim *et al*., 2011). *Labeo rohita* (rohu) is a key Indian major carp in poly-culture systems highly valued for its taste and nutritional benefits (Boonthai *et al*., 2011). Approximately 1.67 million tons of this fast-growing omnivorous fish are produced annually worldwide (Subasinghe *et al*., 2021). Along with being high in protein and n-3 PUFA fatty acids, rohu also contains some calcium and vitamin A (Sharma *et al*., 2023). It is a well-liked option in tropical fish markets due to its great flavor, high market demand, and adaptability. The goal of the current study was to assess how MCP supplementation in a plant meal-based diet affected the rohu fingerlings' ability to grow and digest nutrients.

MATERIALS AND METHODS

Current experimental work was performed in the Fish Nutrition Lab University of Education Lahore. Fingerlings of *L. rohita* were acquired from Fisheries Center Manawa Lahore.

Experimental design

Basal diet was prepared using sesame meal and reference diet with sesame meal being chosen as the test ingredient. Subsequently, the base meal was separated into five test diets and one reference diet having MCP at varying concentrations was supplemented in them (0, 0.20%, 0.40%, 0.60, 0.80%, and 1%). Six fish groups were stocked in tanks and the study was conducted for seventy days.

Experimental procedure

The components were blended into a homogeneous mixture to create six different diets. Each of the six experimental tanks had 100 L of water capacity. The water is kept at a constant 25 ^{o}C. Rohu fingerlings (115) with an average weight of 2 g were acquired from a fish hatchery. For two weeks, the fish were acclimated to the experimental tanks. At the beginning and end of the trial, the fish's weight was recorded.

Formulation of experimental diets

The feed materials were purchased from the nearby commercial market, crashed into a fine powder, and then filtered using a 0.3 mm sieve. The feed components were examined for proximate composition before the experimental diets, were prepared. In all test diets, 1% of Cr_2O_3 was utilized as an inert marker. After adding 10-15% of distilled water feed ingredients were gradually mixed into the mixer to make a dough with the right texture that was pelleted using a pelleting machine. Five MCP-supplemented test diets were prepared and one control diet was formed as represented in Table I.

Sample collection

Following a two-hour feeding period, each tank's valve was opened to remove any leftover feed. To remove the feed particles, the tanks were thoroughly cleaned and more tap water was added. To reduce the quantity of organic constituents that were released into aquatic environment, feces were carefully collected to prevent breaking. To keep the waste for later chemical examination, it was dried in an oven at 65 degrees.

Analyses of growth parameters

To assess the *L. rohita* fingerlings growth performance, the fish juveniles in individual tanks were

Table I. Ingredients composition (%) of control and test diets.

Ingredients	Test diet I	Test diet II	Test diet III	Test diet IV	Test diet V	Test diet VI
	Control	1g	2g	3g	4g	5g
Sesame meal	36	36	36	36	36	36
Fish meal	16	16	16	16	16	16
Rice polish	7	7	7	7	7	7
Wheat bran	9	9	9	9	9	9
Corn glutton 30%	12	12	12	12	12	12
Maize floor	10	9.75	9.5	9.25	9	5
Fish oil	6	6	6	6	6	6
Vitamin-mineral premix	2	2	2	2	2	2
Ascorbic acid	1	1	1	1	1	1
Chronic oxide	1	1	1	1	1	1
MCP	0	0.20	0.40	0.60	0.80	1

* Vit. D3: 3,000,000 IU, Vit. A: 15,000,000 IU Vit. C: 15,000 mg, Vit. B6: 4000 mg, Vit. E:30000 IU. Vit. B2: 7000 mg Vit. B12: 40 mg. Folic acid: 1500 mg, Vit. K3: 8000 mg Ca pantothenate: 12,000 mg, Nicotinic acid: 60,000 mg. Mg: 55 g, Ca: 155 g, Se: 3 mg, Na: 45 g P: 135 g Cu: 600 mg, Mn: 2000 mg, Co: 40 mg, Fe: 1000 mg Zn:3000 mg I: 40 mg. Means within columns with superscript (a) differ considerably at p < 0.05. Data are three replicates' mean (± shows Standard Deviations).

weighed both at the beginning and on termination of the study period. Growth metrics such as the specific growth rate (SGR), weight gain (g), and FCR. Fingerlings' weight increase percentage was determined using standard equations.

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

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

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

Analysis of immunological parameters

Blood samples without an anticoagulant were obtained and kept in a freezer at 20°C for immunological testing. After preparing their smears, centrifugation was used to divide the several smears. Total neutrophils, lymphocytes, monocytes, and eosinophils were determined using the Neubauer differential counting method.

Nutrient absorption

The homogenized samples were oven-dried for 12 hours at 105°C to assess the nutrient digestibility of the excrement and the composition of the test diets. The Kjeldahl apparatus was used to assess the CP levels. In contrast, the Soxhlet system (Soxhlet Extraction Heating Mantels, 250 mL 53868601) using the petroleum ether extraction method was used to measure the crude fat. CHO was calculated by:

$$\text{Total CHO (\%)} = 100 - \text{(all the nutrients)}$$

Table II. Growth parameters of rohu fingerlings fed on MCP supplemented diets.

Test diets	MCP (%)	IW (g)	FW (g)	WG (g)	WG (%)	Wt gain/ day (g)	Feed intake	FCR	Survival (%)	SGR
I	0	6.99 ± 0.38[a]	20.49 ± 0.85[c]	13.50 ± 1.16[c]	193.96 ± 25.58[c]	0.19 ± 0.017[c]	0.31 ± 0.025[a]	1.63 ± 0.030[a]	94.64 ± 0.17[a]	1.20 ± 0.10[c]
II	0.25	6.98 ± 0.35[a]	21.62 ± 0.71[c]	14.64 ± 0.90[c]	210.28 ± 21.70[c]	0.21 ± 0.013[c]	0.33 ± 0.026[a]	1.58 ± 0.037[a]	94.74 ± 5.26[a]	1.26 ± 0.077[bc]
III	0.5	6.98 ± 0.35[a]	24.65 ± 0.82[b]	17.67 ± 1.080[b]	253.99 ± 26.32[ab]	0.25 ± 0.015[b]	0.36 ± 0.026[a]	1.42 ± 0.017[b]	98.15 ± 3.21[a]	1.40 ± 0.085[a]
IV	0.75	7.00 ± 0.37[a]	26.61 ± 0.93[a]	19.62 ± 0.91[a]	280.93 ± 20.23[a]	0.28 ± 0.013[a]	0.36 ± 0.017[a]	1.27 ± 0.013[c]	98.25 ± 3.039[a]	1.48 ± 0.060[a]
V	1	7.023 ± 0.26[a]	23.55 ± 0.58[b]	16.53 ± 0.57[b]	235.61 ± 13.18[bc]	0.24 ± 0.0082[b]	0.34 ± 0.012[a]	1.44 ± 0.064[b]	94.54 ± 0.17[a]	1.34 ± 0.043[ab]
VI	5	7.053 ± 0.26[a]	21.62 ± 0.77[c]	14.57 ± 1.014[c]	207.077 ± 21.29[c]	0.21 ± 0.014[c]	0.34 ± 0.028[a]	1.64 ± 0.069[a]	92.69 ± 3.30[a]	1.24 ± 0.079[bc]
SE		0.06578	0.52956	0.54181	8.46893	0.00774	0.00582	0.03334	0.78868	0.02508
L		0.781	0.004	0.016	0.136	0.016	0.182	0.026	0.509	0.130
Q		0.865	0.000	0.000	0.000	0.000	0.059	0.000	0.039	0.000
Combined		1.000	0.000	0.000	0.003	0.000	0.251	0.000	0.253	0.004

[*a-c] Means within columns having different superscripts are significantly different at $P< 0.05$, Data are means of three replicates (shows Standard Deviations). MCP, Mono-calcium phosphate; IW, initial weight; FW, Final Weight; WG, weight gain; FCR, Feed conversion ratio; SGR, Specific growth rate; Q, Quadratic; L, Linear; C, Combined; Std. E, Standard Error.

Nutrient digestibility coefficient %

Using the standard methodology (NRC, 1993), the apparent nutrient digestibility coefficient (ADC) of the test diets was determine.

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

Statistical exploration

One-way ANOVA was used to evaluate the data on test diet growth metrics, nutrient digestibility, and immunological indices (Steel and Torrie, 1970). The statistical significance of digestibility of nutrients, growth indices and immunology characteristics were examined using the SPSS (Statistical Package for Social Sciences) program. The Duncan test, which was significant at (P< 0.05) was performed to determine whether any significant differences were found.

RESULTS

Growth performance

The growth indices of *L. rohita* fingerlings is shown in Table II and represented in Figure 1. When fish were fed a plant meal-based diet with MCP supplementation, their growth performance was significantly better than that of fish fed a control diet. There was a significant (P< 0.05) increase in weight gain in fish when the MCP concentration increased to a level of 0.60%, however, weight gain was reduced when the MCP level increased further. The fish-fed diet IV (0.60% MCP) had the highest final weight (26.61 g), weight gain (19.62 g), and weight gain (280.93%). In comparison to other test diets, these values were significantly different. The maximum specific growth rate of 1.48 g was also exhibited by group IV. All derived values were statistically similar for the survival rate. When the fish were fed the diet VI (1% MCP), the highest value of FCR (1.64%) was seen, and it was discovered that this value was statistically similar to the fish fed the control diet (0% MCP). The best FCR was recorded at the diet IV with 0.60% MCP supplementation. Fish given the control diet had the lowest FW (20.49 g), WG (13.50 g), and WG% (193.96%) while Group I exhibited the lowest SGR value of 1.20.

Nutrient digestibility

The constituents of the digestible crude protein (CP), ether extract (EE) with gross energy (GE) in the experimental diets for *L. rohita* fingerlings are shown in Tables III-V, and graphically represented in Figure 1B. According to results, it was observed that supplementing a plant-meal based diet with 0.60% MCP significantly improved nutrient digestibility and the least amount of nutrients was released with excrement. All test diets had almost the same CP value, significantly equal amounts of fat, and similar values of GE considering everything. Diet I produced the highest fecal discharge of CP (15.88%) and GE (1.67 Kcal /kg), meanwhile diet VI produced the highest fecal discharge of crude fat (3.68%). The diet IV supplemented with 0.60% MCP showed the lowest discharge of CP (10.74%) and GE (1.16 Kcal /kg). Diet III produced the lowest discharge of crude fat (2.37%). The fish that were fed the test diet IV supplemented with MCP up to 0.60% had the highest CP (67.46%) and GE (68.58 Kcal/kg) digestibility values. The highest crude fat digestibility was observed in the fish that were fed with test diet III with 0.40 MCP supplementation. All things considered, the IV diet showed the best digestibility and the lowest discharge of CP, crude fat, and GE.

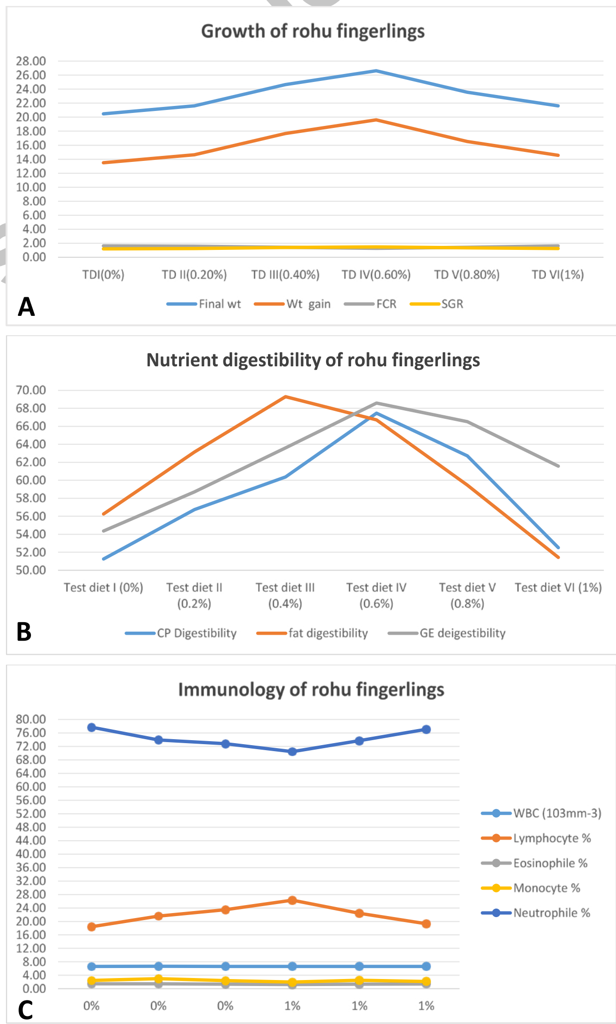

Fig. 1. Effect of HCP supplemented diets on growth performance (A), nutrient digestibility (B) and immunology (C) of rohu.

Table III. Crude protein digestibility of rohu fingerlings fed on MCP supplemented diets.

Test diets	MCP (%)	CP in feces	CP in diet	Crude protein digestibility
I	0	15.88 ± 0.32[a]	30.98 ± 0.35[a]	51.25 ± 0.80[e]
II	0.20	14.27 ± 0.30[b]	30.87 ± 0.41[a]	56.75 ± 0.99[d]
III	0.40	13.12 ± 0.30[c]	30.99 ± 0.50[a]	60.37 ± 0.70[c]
IV	0.60	10.74 ± 0.31[e]	30.98 ± 0.47[a]	67.46 ± 0.84[a]
V	0.80	12.03 ± 0.30[d]	30.96 ± 0.34[a]	62.71 ± 0.78[b]
VI	1	15.49 ± 0.66[a]	30.97 ± 0.48[a]	52.50 ± 0.70[e]
SE		0.4504107	0.0857740	1.3853489
Combined		0.000	0.999	0.000
L		0.000	0.924	0.000

*a-e Means within columns having different superscripts are significantly different at P< 0.05, Data are means of three replicates (shows Standard Deviations). MCP, mono-calcium phosphate; CP, crude protein, Q, quadratic; L, linear; C, Combined; Std. E, Standard Error.

Table IV. Crude fat digestibility of rohu fingerlings fed on MCP supplemented diet.

Test diets	MCP (%)	Fat in feces	Fat in diet	Fat digestibility
I	0	3.31 ± 0.29[b]	7.19 ± 0.44[a]	56.25 ± 0.94[e]
II	0.20	2.84 ± 0.14[cd]	7.20 ± 0.31[a]	63.16 ± 0.96[c]
III	0.40	2.37 ± 0.15[e]	7.21 ± 0.24[a]	69.29 ± 0.89[a]
IV	0.60	2.55 ± 0.23[de]	7.18 ± 0.45[a]	66.70 ± 0.95[b]
V	0.80	3.04 ± 0.22[bc]	7.19 ± 0.35[a]	59.47 ± 0.85[d]
VI	1	3.68 ± 0.18[a]	7.21 ± 0.37[a]	51.42 ± 0.99[f]
SE		0.1152781	0.0729695	1.4888265
Combined		0.000	1.000	0.000
L		0.020	0.988	0.000

*a-f Means within columns having different superscripts are significantly different at P< 0.05, Data are means of three replicates (shows Standard Deviations). MCP, Mono-calcium phosphate; Crude fat, Q-Quadratic; L, Linear; C, Combined; Std. E, Standard Err.

Immunology

The results of the immunological parameter of *L. rohita* fingerlings fed on a plant-meal based diet supplemented with MCP are exhibited in Table VI and Figure 1C. The data in the table demonstrated that there is no discernible variation in the counts of WBC and eosinophils in all diets. The WBCs values were significantly similar for all test diets. Regarding lymphocytes, the IV diet yielded the highest count (26.32%), while the control diet produced the lowest level (18.40%). When it came to monocyte count, fish fed the diet II had the highest value (2.99%), whereas fish-fed diet IV had the lowest value (1.98%).

Table V. GE digestibility of rohu fingerlings fed on the MCP supplemented diet.

Test Diets	MCP (%)	GE in feces	GE in diet	GE digestibility
I	0	1.67 ± 0.07[a]	3.49 ± 0.21[a]	54.35 ± 0.94[f]
II	0.20	1.55 ± 0.15[ab]	3.51 ± 0.24[a]	58.71 ±0.81[e]
III	0.40	1.35 ± 0.10[cd]	3.46 ± 0.17[a]	63.60 ±0.93[c]
IV	0.60	1.16 ± 0.06[d]	3.47 ± 0.18[a]	68.58±0.580[a]
V	0.70	1.22 ± 0.11[cd]	3.48 ± 0.23[a]	66.52± 0.640[b]
VI	1	1.41 ± 0.10[bc]	3.48 ± 0.18[a]	61.57± 0.92[d]
SE		0.0478256	0.0406441	1.1638249
Combined		0.000	1.000	0.000
L		0.000	0.897	0.000

*a-f Means within columns having different superscripts are significantly different at *P*< 0.05, Data are means of three replicates (shows Standard Deviations). MCP, Mono-calcium phosphate; GE, Gross energy; Q, Quadratic; L, Linear; C, Combined; Std. E, Standard Error.

Table VI. Immunology of rohu fingerlings fed with MCP supplemented diet.

Test diets	MCP (%)	WBCs	Lymphocyte %	Eosinophil %	Monocyte %	Neutrophyls %
1	0	6.62 ± 0.42[a]	18.40 ± 0.82[d]	1.45 ± 0.28[a]	2.45 ± 0.28[b]	77.70 ± 0.73[a]
2	0.20	6.67 ± 0.28[a]	21.59 ± 0.90[c]	1.48 ± 0.19[a]	2.99 ± 0.24[a]	73.93 ± 0.95[b]
3	0.40	6.63 ± 0.24[a]	23.46 ± 0.75[b]	1.34 ± 0.12[a]	2.39 ± 0.25[b]	72.81 ± 0.57[b]
4	0.60	6.65 ± 0.40[a]	26.32 ± 0.83[a]	1.24 ± 0.15[a]	1.98 ± 0.19[c]	70.46 ± 0.77[c]
5	0.80	6.64 ± 0.33[a]	22.41 ± 0.89[bc]	1.36 ± 0.11[a]	2.53 ± 0.16[b]	73.70 ± 0.76[a]
6	1	6.64 ± 0.25[a]	19.32 ± 0.99[d]	1.43 ± 0.081[a]	2.16 ± 0.18[bc]	77.09 ± 1.24[a]
SE		0.06489	0.65829	0.03870	0.08830	0.62469
Combined		1.000	0.000	0.554	0.002	0.000
L		0.987	0.036	0.510	0.011	0.167
Q		0.917	0.000	0.196	0.978	0.000

*a-d Means within columns having different superscripts are significantly different at *P*< 0.05, Data are means of three replicates (shows standard deviations). MCP, Mono-calcium phosphate; WBCs, white blood cells; Q, Quadratic; L, Linear; C, Combined; Std. E, Standard error.

DISCUSSION

In the current investigation diets based on sesame meal that were supplemented with MCP at graded levels (0, 0.20%, 0.40%, 0.60, 0.80%, 1%) resulted in better weight gain, weight increase percentage, and FCR when compared to the control diet. At 0.60% MCP supplementation, the largest increase in weight gain was observed. It indicates that sesame meal supplemented with MCP has the potential to be an alternative protein source of fish meal.

Similarly, Kim *et al*. (1998) observed that the ideal MCP level for mirror carp was 0.75 of available P. This resulted in better weight gain, maximal growth, and FCR. Andrew *et al*. (1973) observed comparable outcomes. The study findings indicated that there was a substantial increase in growth and survival rate ($P< 0.05$) when the phosphorus content in the diet was raised to 0.75% and 1.5% of the calcium. Nearly similar to current results Hernandez *et al*. (2005) discovered that rainbow trout grew more quickly and gained more weight and feed conversion ratio when fed diets supplemented with 0.5% MCP as a P supplement. Slightly different from present results Abdel *et al*. (2018) concluded that the greatest outcomes come from using MCP as a phosphorus supply at the ideal level of 0.9%. Different from my findings Robinson *et al*. (1987) determined that the inclusion of 0.30% dietary P and 0.70% dietary calcium were sufficient for healthy growth. Growth differences were not observed in fish-fed diets containing 0.70-1.00% dietary calcium. In contrast to these findings, Chinkichi and Hiroshi (1976) noted that the diets provided to the fish varied in terms of calcium and phosphorus content. The greatest weight gain was obtained at a phosphorus level of 0.7%, but the growth rate was unaffected by the level of calcium. In contrast to my research work Fontagne *et al*. (2009) conducted an experiment and found that the group with a high P level of 1.6% had the lowest survival rate. The addition of calcium to the diet did not appreciably affect fish survival. In opposition to my study, Jin *et al*. (2012) revealed that the fish's growth was unaffected at low inclusion P levels, but that the sturgeon's total length showed negative growth when the dietary P content reached 1.85%. In contrast to current research work Kim and Ahn (1993) observed that the feed conversion ratio, protein efficiency ratio, and daily growth index increased by around two times at 2% MCP. In contrast to my studies Chen *et al*. (2018) discovered that the MCP source's 5.9 g/kg of accessible P resulted in the highest weight gain and the lowest feed conversion ratio. The explanation for the variation in the results is unclear however, it could be caused by various fish species, feed components, experimental setups feed processing techniques, or feed drying techniques. The results of the current study indicate that as compared to the control diet (which did not include MCP supplementation), the addition of MCP to a plant meal-based diet improved the ADC% of nutrients for *L. rohita* fingerlings. Fish feeding at 0.60% of MCP-supplemented plant meal-based diet showed the highest values for ADC% of CP and GE. Unlike my research Wang *et al*. (2022) concluded that 1.5% MCP supplementation produced the highest value of protein retention and ADC of CP. Kim and Ahn (1993) concluded that the apparent digestibility coefficients of nutrients by the two diets one with 2% MCP-supplementation and the other without MCP did not differ considerably. In comparison to the results of the current work, the results were significantly different. However, Morales *et al*. (2018) found no significant effect on nutrient digestibility in fish fed on plant-based test diets supplemented with MCP as a phosphorus source. Hossain *et al*. (2020) in his research work observed that fish fed with the MCP supplemented diet showed lower phosphorus and calcium digestibility as compared to the other phosphorus sources. Variations in the amount of MCP in feed ingredients, the type of fish utilized, and feed ingredient properties could account for discrepancies in the findings of different researchers. The highest levels of WBCs, lymphocytes, and eosinophils were observed in the current study at 0.60 MCP-supplemented plant meal-based diet. MCP supplementation was recently suggested as a fish immune system stimulant. In contrary to present findings Abdel *et al*. (2018) several sources of phosphorus represent the levels of WBC and hematological indicators. The results showed that WBCs had the maximum value with 0.9% MCP supplementation. Nevertheless, this investigation did not document other immunological indices. Unlike my findings, an investigation by Hossain *et al*. (2020) revealed that phosphorus from the MCP source did not enhance the fish's immunity when compared to other sources. When compared to the control diet in the current study, the addition of MCP significantly improved the nutrient digestibility, growth performance, and immunological indicators of *L. rohita* fingerlings. Additionally, it was discovered that the ideal MCP supplementation amount in a plant meal-based diet for the greatest enhancement of fish performance is 0.60%.

Few studies on immunology and nutrient digestibility with MCP supplementation in plant-based diets have been conducted. Additional research is necessary to completely comprehend the effects of MCP supplementation on fish immunity.

The pollution of aquatic environments poses significant risks to fish health and survival, with contaminants inducing oxidative stress and various physiological disturbances Dastan *et al*. (2014). These stressors negatively affect critical functions, stunting growth and immune responses in fish. Antioxidative agents like selenium and propolis have shown promise in mitigating oxidative damage from pollutants Gulhan *et al*. (2012). Implementing plant-based diets enriched with nutrients such as MCP offers a sustainable approach to counteract these adverse effects, enhancing fish growth and resilience against environmental challenges Tales *et al.* (2014). By promoting balanced nutrition and addressing pollution's impact, aquaculture can sustainably yield nutrient-rich aquatic products while aligning with environmental conservation goals.

The role of environmental management in aquaculture protecting aquatic ecosystems from pollution is essential for the sustainability of aquaculture and the welfare of aquatic species. Regulatory frameworks aimed at minimizing harmful emissions and promoting eco-friendly practices can significantly reduce oxidative stress and improve fish health (Caglar *et al*., 2019; Selamoglu *et al*., 2015). Additionally, incorporating cold chain logistics in the marketing of aquatic products enhances quality and safety for consumers, as noted by Selamoglu (2021b). This research underscores the importance of a holistic approach to aquaculture management, blending innovative dietary strategies with robust environmental management to protect fish health and ensure the sustainability of aquatic ecosystems.

CONCLUSION

Based on the current research, it is concluded that supplementing a plant meal based diet with 0.60% MCP improves the performance and overall health of rohu fingerlings by improving their absorption of essential nutrients, which promotes better growth.

DECLARATIONS

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Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdel-Bary, D.A., Khattab, H.M., Amer, M.A. and Hashim, A., 2018. Effect of some major elements on growth performance and blood parameters in fish. *Arab Univ. J. agric. Sci.*, **26**: 2325-2334. https://doi.org/10.21608/ajs.2018.35558

Acar, Ü. and Türker, A., 2018. The effects of using peanut meal in rainbow trout (*Oncorhynchus mykiss*) diets on the growth performance and some blood parameters. *Aquacult. Stud.*, **18**: 5-13.

Agrahar, M.D., Dwivedi, S., Dixit-Bajpai, P. and Kumar, M., 2018. Effect of natural fortification with calcium and protein rich ingredients on texture, nutritional quality and sensory acceptance of cookies. *Nutr. Fd. Sci.*, **48**: 807-818. https://doi.org/10.1108/NFS-02-2018-0041

Akter, T., Hossain, A., Rabiul Islam, M., Hossain, M.A., Das, M., Rahman, M.M. and Abdel-Tawwab, M., 2023. Effects of spirulina (*Arthrospira platensis*) as a fishmeal replacer in practical diets on growth performance, proximate composition, and amino acids profile of pabda catfish (*Ompok pabda*). *J. appl. Aquacult.*, **35**: 69-82. https://doi.org/10.1080/10454438.2021.1936740

Amescua, A., 2023. Marine fishing and aquaculture: A global perspective. In: *Farm Animal Welfare Law.* CRC Press. pp. 11-34. https://doi.org/10.1201/9781003137733-3

Andrews, J.W., Murai, T. and Campbell, C., 1973. Effects of dietary calcium and phosphorus on growth, food conversion, bone ash and hematocrit levels of catfish. *J. Nutr.*, **103**: 766-771. https://doi.org/10.1093/jn/103.5.766

Ates, B., Orun, I., Talas, Z.S., Durmaz, G. and Yilmaz, I., 2008. Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) exposed to Pb^{2+} and Cu^{2+}. *Fish Physiol. Biochem.*, **34**: 53-59. https://doi.org/10.1007/s10695-007-9146-5

Ayoola, A., 2010. *Replacement of fishmeal with alternative protein sources in aquaculture diets*. pp. 1-145. http://www.lib.ncsu.edu/resolver/1840.16/6546

Boonthai, T., Vuthiphandchai, V. and Nimrat, S., 2011. Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaeus monodon*). *Aquacult. Nutr.*, **17**: 634-644. https://doi.org/10.1111/j.1365-2095.2011.00865.x

Caglar, M., Canpolat, O. and Selamoglu, Z., 2019. Determination of some heavy metal levels in three freshwater fish in Keban Dam Lake (Turkey) for public consumption. *Iran. J. Fish. Sci.,* **18**: 188–198.

Chakraborty, P., Mallik, A., Sarang, N. and Lingam, S.S., 2019. A review on alternative plant protein sources available for future sustainable aqua feed production. *Int. J. chem. Stud.*, **7**: 1399-1404.

Chen, A., Bu, X., Yu, S., Luo, C., Wang, Y., Zhou, Q. and Yang, Y., 2018. Optimum level of dietary monocalcium phosphate determined based on optimal growth and vertebrae phosphate content of juvenile Ussuri catfish, *Pseudobagrus ussuriensis*. *Aquacult. Nutr.*, **24**: 1484-1493. https://doi.org/10.1111/anu.12685

Chinkichi, O. and Hiroshi, T., 1976. Mineral requirements in fish. III. Calcium and phosphorus requirements in carp. *J. Japan. Soc. Fish. Sci.,* **7**: 793-99. https://doi.org/10.2331/suisan.42.793

Costa, J.M., Sartori, M.M., Nascimento, N.F.D., Kadri, S.M., Ribolla, P.E., Pinhal, D. and Pezzato, L.E., 2018. Inadequate dietary phosphorus levels cause skeletal anomalies and alter osteocalcin gene expression in zebrafish. *Int. J. mol. Sci.*, **19**: 364. https://doi.org/10.3390/ijms19020364

Dastan, S.D., Dastan, T., Gulhan, M.F., KiRkbes, A. and Talas, Z.S., 2014. Biochemical changes in muscle and gill tissues of rainbow trout treated with various concentrations of pollen extract. *Res. Opin. Anim. Vet. Sci.*, **4**: 540–544. https://www.cabdirect.org/abstracts/20143317455.html

Eniayejuni, A., 2020. Scientific research in west Africa and the impact of international collaboration: An analysis in scopus database, 1997-2017. *Afr. J. Libr. Arch. Inf. Sci.*, **30**: 1-15. http://www.ajol.info/journals/ajlais

F.A.O., 2011. Aquaculture department. 2013. *Glob. Aquacult. Prod. Stat. Year*, **495**: 568-574.

F.A.O., 2018. *The state of world fisheries and aquaculture*. Meeting the sustainable development goals.

F.A.O., 2017. *A tool for fishery statistics analysis*. FAO Fisheries and Aquaculture Department, FIPS–Statistics and information: Rome, Italy.

Fontagné, S., Silva, N., Bazin, D., Ramos, A., Aguirre, P., Surget, A. and Power, D.M., 2009. Effects of dietary phosphorus and calcium level on growth and skeletal development in rainbow trout (*Oncorhynchus mykiss*) fry. *Aquaculture*, **297**: 141-150. https://doi.org/10.1016/j.aquaculture.2009.09.022

Gulhan, M.F., Duran, A., Talas, Z.S., Kakoolaki, S. and Mansouri, S.M., 2012. Effects of propolis on microbiologic and biochemical parameters of Rainbow trout (*Oncorhynchus mykiss*) after exposure to the pesticide. *Iran. J. Fish. Sci.*, **11**: 490–503.

Hardy, R.W., 2010. Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquacult. Res.*, **41**: 770-776. https://doi.org/10.1111/j.1365-2109.2009.02349.x

Hassan, M.S., Soltan, M.A., Mohammady, E.Y., Elashry, M.A., El-Haroun, E.R. and Davies, S.J., 2018. Growth and physiological responses of Nile tilapia, *Oreochromis niloticus* fed dietary fermented sunflower meal inoculated with *Saccharomyces cerevisiae* and *Bacillus subtilis*. *Aquaculture*, **495**: 592-601. https://doi.org/10.1016/j.aquaculture.2018.06.018

Hernández, A., Satoh, S. and Kiron, V., 2005. Effect of monocalcium phosphate supplementation in a low fish meal diet for rainbow trout based on growth, feed utilization, and total phosphorus loading. *Fish. Sci. Res.*, **71**: 817-822. https://doi.org/10.1111/j.1444-2906.2005.01032.x

Hossain, M.S., Chance, A.B., El, N., Wattiez, X., Houndji, A., Mandiki, S.N. and Kestemont, P., 2020. Dietary inorganic monophosphates in high plant ingredient-based diets influence nutrient digestibility, postprandial macro-mineral status and immune functions of juvenile rainbow trout, *Oncorhynchus mykiss*. *Aquacult. Nutr.*, **26**: 2178-2194. https://doi.org/10.1111/anu.13156

İbrahim, O., Zeliha, S. and Aysel, A.U., 2011. Modulating effect of selenium on gills of fish exposed to heavy metals. *Fresenius Environ. Bull*., 20(1): 104-108.

Jin, J.L., Wang, C.F., Tang, Q., Xie, C.X. and Dai, Z.G., 2012. Dietary phosphorus affected growth performance, body composition, antioxidant status and total P discharge of young hybrid sturgeon (♀ Huso huso×♂ Acipenser schrenckii) in winter months. *J. appl. Ichthyol.*, **28**: 697-703. https://doi.org/10.1111/j.1439-0426.2012.02024.x

Kakoolaki, S., Talas, Z.S., Cakir, O., Ciftci, O. and Ozdemir, I., 2013. Role of propolis on oxidative stress in fish brain. *PubMed*., https://pubmed.ncbi.nlm.nih.gov/25337342

Kim, J.D. and Ahn, K.H., 1993. Effects of mono calcium phosphate supplementation on phosphorus discharge and growth of carp (*Caprinus carpio*) grower. *Asian Austral. J. Anim. Sci.*, **6**: 521-526. https://doi.org/10.5713/ajas.1993.521

Kim, J.D., Kim, K.S., Song, J.S., Lee, J.Y. and Jeong, K.S., 1998. Optimum level of dietary monocalcium phosphate based on growth and phosphorus excretion of mirror carp, *Cyprinus carpio*. *Aquaculture*, **161**: 337-344. https://doi.org/10.1016/S0044-8486(97)00281-0

Mallioris, P., Kotzamanis, Y., Vardali, S., Roussos, E., Ilia, V., Paschali, E. and Vatsos, I.N., 2022. Modulation of intestinal health and hepatic vacuolation in gilthead sea bream (*Sparus aurata*) juveniles by a mixture of dietary esterified butyrins, emulsifiers from plants and yeast extracts at low and high fish meal inclusion. *Anim. Feed Sci. Technol.*, **284**: 115194. https://doi.org/10.1016/j.anifeedsci.2021.115194

McNicoll, G., 1992. The United Nations long-range population projections. *PopDev.*, **18**: 333-340. https://doi.org/10.2307/1973683

Morales, G.A., Azcuy, R.L., Casaretto, M.E., Márquez, L.,Hernández, A.J., Gómez, F. and Mereu, A., 2018. Effect of different inorganic phosphorus sources on growth performance, digestibility, retention efficiency and discharge of nutrients in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **495**: 568-574. https://doi.org/10.1016/j.aquaculture.2018.06.036

NRC, 1993. *Nutrients requirements of fish*. National Academy Press, Washington, DC USA.

Ogino, S. and Takeda, H., 1976. Mineral requirements in fish. III. Calcium and phosphorus requirements in carp. *Bull. Japan Soc. Sci. Fish.*, **42**: 793-799. https://doi.org/10.2331/suisan.42.793

Onsaard, E., Pomsamud, P. and Audtum, P., 2010. Functional properties of sesame protein concentrates from sesame meal. *Asian J. Fd. Agro-Indust.*, **3**: 420-431.

Rao, J., Garfinkel, C.I. and White, I.P., 2020. Predicting the downward and surface influence of the February 2018 and January 2019 sudden stratospheric warming events in subseasonal to seasonal (S2S) models. *J. Geophys. Res. Atmos.*, **125**: e2019JD031919. https://doi.org/10.1029/2019JD031919

Resilience, B., 2017. The state of food security and nutrition in the world. *Food and Agriculture Organization of the United Nations*, pp. 1-132.

Robinson, E.H., LaBomascus, D., Brown, P.B. and Linton, T.L., 1987. Dietary calcium and phosphorus requirements of *Oreochromis aureus* reared in calcium-free water. *Aquaculture*, **64**: 267-276. https://doi.org/10.1016/0044-8486(87)90189-X

Selamoglu, M., 2021a. Blue economy and blue ocean strategy. *J. Ecol. Natl. Resour.*, **5**. https://doi.org/10.23880/jenr-16000263

Selamoglu, M., 2021b. The effects of the ports and water transportation on the aquatic ecosystem. *Open Access J. Biogener. Sci. Res.*, **10**. https://doi.org/10.46718/JBGSR.2021.10.000239

Selamoglu, Z., 2020. Zooplankton diversity of three dam lakes in Turkey. *Iran. J. Fish. Sci.,*

Selamoglu, Z., Duran, A., Gulhan, M. and Erdemli, M., 2015. Effects of propolis on biochemical and microbiological parameters in carp (*Cyprinus carpio*) fillets exposed to arsenic. *Iran. J. Fish. Sci.*, **14**: 896–907. http://agrijournals.ir/article_114491.

Sharma, P., Joshi, R., Ciji, A., Akhtar, M.S. and Sarma, D., 2023. Nutritional quality and human health benefits of important cold-water fishes of the Indian Himalayas. In: *Fish. Aquacult. Tem. Himalayas*, Singapore: Springer Nature Singapore. pp. 341-370. https://doi.org/10.1007/978-981-19-8303-0_19

Steel, R.G. and Torrie, J.H., 1970. *Principles and procedures of statistics*. McGraw-Hill Education.

Subasinghe, R., Baron, P.J., Beveridge, M., Marschoff, E.R. and Oliva, D., 2021. *Changes in aquaculture*. https://hdl.handle.net/10037/19004

Subasinghe, R., Soto, D. and Jia, J., 2009. Global aquaculture and its role in sustainable development. *Rev. Aquacult.*, **1**: 2-9. https://doi.org/10.1111/j.1753-5131.2008.01002.x

Talas, Z., Gulhan, M., Erdogan, K. and Orun, I., 2014. Antioxidant effects of propolis on carp *Cyprinus carpio* exposed to arsenic: Biochemical and histopathologic findings. *Dis. Aquat. Organ.*, **108**: 241–249. https://doi.org/10.3354/dao02714

Torstensen, B.E., Espe, M., Sanden, M., Stubhaug, I., Waagbø, R., Hemre, G.I. and Berntssen, M.H.G., 2008. Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture*, **285**: 193-200. https://doi.org/10.1016/j.aquaculture.2008.08.025

Uniyal, S., Paliwal, R., Kaphaliya, B. and Sharma, R.K., 2020. Human overpopulation: Impact on environment. In: *Megacities and rapid urbanization: Breakthroughs in research and practice*, pp. 20-30. IGI Global. https://doi.org/10.4018/978-1-5225-9276-1.ch002

Wang, P., Li, X., Xu, Z., Ji, D., He, M., Dang, J. and Leng, X.J., 2022. The digestible phosphorus requirement in practical diet for largemouth bass (*Micropterus salmoides*) based on growth and feed utilization. *Aquacult. Fish.*, **7**: 632-638. https://doi.org/10.1016/j.aaf.2020.11.002