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RESEARCH ARTICLE

Development of Cost Effective and Eco-Friendly Fish Feed by Using Di Calcium Phosphate Supplemented Plant Meal Based Diet for *Cirrhinus mrigala* (Mori) Juveniles

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ABSTRACT

The goal of current study is to provide a cost-effective and environmentally sustainable diet for *Cirrhinus (C.) mrigala* fingerlings by incorporating Di Calcium Phosphate (DCP) into a plant-based diet to improve hematological indices, nutrient digestibility, and mineral absorption. Six test meals (0, 1, 2, 3, 4, 5) were created using different DCP levels (0, 0.20, 0.40, 0.60, 0.80 and 1.00 g/Kg). 20 fingerlings were placed in each glass tank for the trial and were fed twice daily. Values were subjected to one-way analysis of variance by applying Duncan's test to find out the statistics of nutrient digestibility, mineral absorption, hematological indices and considered significant at P<0.05. Result showed that statistically improved values of RBCs (3.05×10^6 mm^{-3}), Hb (8.01 g/100mL), and Ht (32.47%) were computed at diet 3 having 0.60 g/kg level of DCP, whereas test diet 2 (0.40g/kg) had the maximum value of PLT (63.40%). Significantly, the highest values of crude protein (70%) and fat (69%) observed at test diet 3 having 0.60g/kg level of DCP but, gross energy (68%) was observed at test diet 2 having (0.40g/kg DCP level). At test diet 3 (0.60g/kg), mineral absorption was likewise at its maximum value (Ca, Na, K, P as 72.21, 72.45, 66.55 and 72.47%, respectively). Compared to the control group and all other test diets, all of these values were significantly different. Based on these results, it can be concluded that, diet 3 (0.60g/kg) reduces the discharge of minerals and nutrients into water with improved hematological indices, as it was the optimal DCP level for producing economical and environment friendly feed for *C. mrigala* fingerlings.

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INTRODUCTION

Food insecurity has become a serious problem due to increasing food consumption brought on by overcrowding and rapid population expansion (Yasmine *et al.,* 2023). Aquaculture feed industry, which expanded at an average annual rate of 5.8% between 2001 and 2016, the subject of research (Rao *et al*., 2019). Fish feed is a major expense, contributing up about half of total costs. To avoid ecological restrictions, nutrient-dense, sustainable foods are essential. Fish meal (FM), which provides vital nutrients for proper body development, is necessary for aquaculture diets. As demand has increased, prices have risen steadily (Zaniboni-Filho *et al.,* 2018). The fish industry's goal is to create high-quality, affordable fish feed, because FM has a high protein content and an amino acid profile, it is the main ingredient. (Bloecher *et al.*, 2021).

Alternate sources of protein other than fish meal are needed for aquaculture sustainability. The importance of replacing FM with various plant protein sources is emphasized (Hussain *et al*., 2024). A shortage of raw materials and an inconsistent supply provide challenges to the production of FM (FAO, 2018). Choosing aqua feed made from either plants or animals might save money (Mahboob, 2014). Plant sources of protein are more widely available and less expensive than animal-derived alternatives for fish feed. They contain higher levels of protein and nutrients than proteins derived from animals (Li *et al.,* 2024). Plants such as linseed, sunflower, soybean, and blackseed, as well as their pods, grains, leaves, and seeds are excellent sources of plant material for fish diet (Hekmatpour *et al.*, 2023). Feed cost-effectiveness may be raised, and waste can be decreased by formulating feeds with digestible P levels that correspond to fish requirements (Pimentel-Rodrigues and Oliva-Teles, 2001).

The fish *C. mrigala* ranks third among the principal carps of continent, behind Bhakur (*Catla catla*) and Rohu (*Labeo rohita*) (Mishra, 2020). Diets for *C. mrigala* often consist of a mix of other feed items and different sources of protein. Due to this, the aquaculture system's nitrogen excretion increased, nitrogen consumption became unbalanced, and feed costs increased. Given that different developmental stages have different food needs, the basic protein requirement of *C. mrigala* is 28 to 33% (Alam, 2020).

The use of dicalcium phosphate in aquaculture is essential for the growth, health, and reproduction of fish. It is a cost-effective alternative to other sources of phosphorus, and it has numerous benefits. It promotes the growth of fish by providing essential minerals and phosphorus. It enhances the immunity of fish, making them less susceptible to diseases (Dwivedi *et al.*, 2016). In order to evaluate the effects of DCP supplements on the haematology, mineral absorption, and nutritional digestibility of Mori fingerlings fed a diet based primarily on plant by products that is both economical and environmentally suitable, the current study was designed.

MATERIALS AND METHODS

Study site and experimental conditions: The experiment was performed at the University of Education's Township Campus in Lahore, Pakistan's Fish Nutrition Laboratory, Department of Zoology, Division of Science & Technology. To this end, *C. mrigala* fingerlings (120) were purchased from the Manawa Fish Hatchery in Lahore having an average weight of 2g. The fingerlings were acclimated in the lab environment for two weeks. Each aquarium with a 70-liter water holding capacity was set up in order to stock fingerlings. After dichlorination, the water was aerated. The water was always kept at a temperature of 25°C. Before the experiment began, *C. mrigala* fingerlings were treated with salt solution for two to three minutes to get rid of any parasites. A diet based on plant meals supplemented with DCP served as the test element for experimental diet. The fish were fed for a total of eight weeks. The fish's weight was recorded at the start and end of the experiment.

Sesame (Til) meal was purchased from the local market of Lahore and used in our prepared test diets as compared with the fish meal. But as per chemical analysis, it was found that there were lower contents of phosphate and calcium in sesame meal that were covered with the help of supplementation of DCP. The DCP was used in a very minute quantity that was about 0.60% for maximum results (240g for 40kg diet). To supply air through the capillary system, an air pump was used throughout the duration of the experiment. Regular observations were made of the typical range of water quality measures, such as pH, dissolved oxygen, and temperature. The fish were fed for 70 days throughout this time.

Formulation of feed pellets: All feed ingredients (Table 1) were bought from the Lahore local market. The ingredients were well mixed for five to ten minutes in a blender. As the mixture was being combined, fish oil and distal water were progressively added. The mixture of all ingredients was run through a mincer machine to create pellets with a diameter of 2mm. After that, the pellets were dried in a drying chamber at 40°C until the moisture content was roughly 10%. The pellets were dried, then crushed into the appropriate sizes and stored at 4°C.

Feeding and Sample Collection: The *C. mrigala* fingerlings were fed 4% of their live, wet body weight twice a day according to the prescribed diet. Remaining food was removed from the tanks after a two-hour feeding period. The tanks were completely cleaned to remove any remaining feed material before fresh water was supplied. Next, collect excrement so that it doesn't break. After three to four hours of drying at 65°C in an oven, feces samples were kept at room temperature in preparation for chemical analysis.

Hematological indices: Anesthesia was given to the fish, and the caudal vein was punctured using a heparinized syringe. Hematological indices were examined in a lab environment using these blood samples. Hematocrit was analyzed using capillary tubes and the micro-hematocrit technique. Red blood cell counts were conducted using hemocytometers equipped with authorised Neubauer counting chambers. The description was used to calculate the concentration of hemoglobin, or Hb.
The following formulas were used, in that order, to compute MCHC, MCH, and MCV:

$$MCHC = Hb / PCV \times 100$$
$$MCH = Hb / RBC \times 10$$
$$MCV = PCV / RBC \times 10$$

Digestibility of Nutrients: Samples of homogenized food and excrement were dried in an oven at 105°C for almost 12 hours, and measurements were made of the moisture content of the excrement and test diets. The CP contents of the feed and feces were identified using the Micro Kjeldahl device. The petroleum ether extraction method was employed to examine the EE utilizing the Soxhlet apparatus. Estimating the sample of GE was done using the oxygen bomb calorimeter. For the calculation, the following formula was used.

$$\text{Total carbohydrate (\%)} = 100-(\text{Moisture\%} + \text{EE\%} + \text{CP\%} + \text{Ash\%} + \text{CF \%})$$

Assessment of minerals: In order to evaluate the minerals (Ca, P, Cu and Mg) in prepared feces and food samples, the Samples underwent atomic absorption in accordance with the AOAC, (1995) procedure. The potassium and sodium percentages in the sample were measured using flame photometry (Jenway PFP-7, UK). The samples, which served as markers, had their chromic oxide fractions oxidized with a molybdate reagent. Then, the samples' absorbance was measured at a wavelength of 370nm using a UV-VIS 2001 Spectrophotometer. Once more, a spectrophotometer was used to measure the absorbance at 720nm wavelength in order to quantify the phosphorus concentrations in the samples.

Table 1: Ingredients/chemical composition (%) of test diet

Ingredients	Control	TD-I	TD-II	TD-III	TD-IV	TD-V
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 Gluton 30%	12	12	12	12	12	12
Maize flour	10	9.80	9.60	9.40	9.20	9
Fish oil	6	6	6	6	6	6
Vitamin/Mineral Premix	2	2	2	2	2	2
Ascrobic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
DCP	0	0.20	0.40	0.60	0.80	1
Mineral composition in feed						
Ca	0.74±0.08[a]	0.74±0.07[a]	0.75±0.09[a]	0.76±0.12[a]	0.75±0.12[a]	0.77±0.08[a]
Na	0.063±0.50[a]	0.067±0.050[a]	0.057±0.031[a]	0.020±0.60[a]	0.0253±0.040[a]	0.067±0.05[a]
K	1.35±0.06[a]	1.34±0.05[a]	1.36±0.05[a]	1.35±0.08[a]	1.34±0.06[a]	1.35±0.06[a]
P	1.98±0.08[a]	1.98±0.09[a]	1.98±0.09[a]	1.99±0.08[a]	1.97±0.10[a]	1.98±0.09[a]
Fe	0.057±0.07[a]	0.057±0.029[a]	0.050±0.05[a]	0.067±0.70[a]	0.550±0.60[a]	0.70±0.66[a]
Cu	0.49±0.08[a]	0.049±0.09[a]	0.049±0.08[a]	0.049±0.08[a]	0.048±0.06[a]	0.049±0.05[a]
Mg	0.040±0.05[a]	0.040±0.03[a]	0.040±0.08[a]	0.038±0.05[a]	0.037±0.06[a]	0.039±0.06[a]
Al	0.040±0.05[a]	0.040±0.003[a]	0.040±0.08[a]	0.038±0.05[a]	0.037±0.06[a]	0.039±0.06[a]
Zn	0.07±0.06[a]	0.07±0.06[a]	0.08±0.07[a]	0.09±0.07[a]	0.04±0.07[a]	0.03±0.06[a]
Nutrient composition in Feed						
Crude Protein%	30.98±0.35[a]	30.87±0.41[a]	30.99±0.50[a]	30.98±0.47[a]	30.96±0.34[a]	30.97±0.48[a]
Crude fat %	7.19±0.44[a]	7.20±0.31[a]	7.21±0.24[a]	7.18±0.45[a]	7.19±0.35[a]	7.21±0.37[a]
Gross energy (kcalg-1)	3.49±0.21[a]	3.51±0.24[a]	3.51±0.24[a]	3.47±0.18[a]	3.48±0.23[a]	3.48±0.18[a]

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

Calculation of Apparent Digestibility Coefficient (ADC %): NRC (1993) standard formula was used to calculate the apparent digestibility coefficient % of nutrients for all meals in the test diets.

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

Statistical analysis of the samples: The apparent digestibility constant of the nutrient's digestibility, hematological indices, and mineral absorption were calculated using one-way ANOVA. Using the Statistical Package for Social Science (SPSS) Duckan test, the mean variations were assessed for significance at P<0.05. We utilized SPSS for statistical analysis.

RESULTS

Hematological indices: In this study, table 2 displayed the effect of di-calcium phosphate supplemented in plant-based diet on hematocrit (Ht), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), and red blood cells (RBC) of mori fingerlings. Best hematological indices were observed at test diet 3 having 0.60% DCP inclusion.

The highest values of RBC (3.05), Hb (8.01%), PCV (28.69%), MCHC (37.59%) and Ht (32.47%) were observed at test diet 3 having 0.60 % DCP level. Whereas the second highest value of RBC (2.81), PCV (27.54%) and MCHC (36.66%) were observed in test diet 2 having 0.40 % level of DCP as presented in Fig. 1. These values were significantly similar to each other. Fingerlings fed on test diet 4 had the second-highest values of Hb (7.58%) and Ht (29.43%), with a 0.80% DCP level. There was a significant difference between these values. Maximum PLT values (63.40) were observed at test diet 2 having 0.40% DCP inclusions while second highest (62.52) values were noticed at test diet 3 having 0.60% of DCP. Both values were non-significant to each other. Lowest values of RBCs (1.61), PLT (53.49), Hb (5.03%), PCV (21.36), MCH (36.28), MCV (96.39) and Ht (21.51%) were observed at control diet having no DCP supplementation. All these values differ significantly from each other while lowest value of MCHC (30.72%) was found in the test diet 5 having 1% DCP level. In combined, linear and quadratic tests values of RBCs, Hb, Ht and MCV were significantly differ from each other and from other test diets.

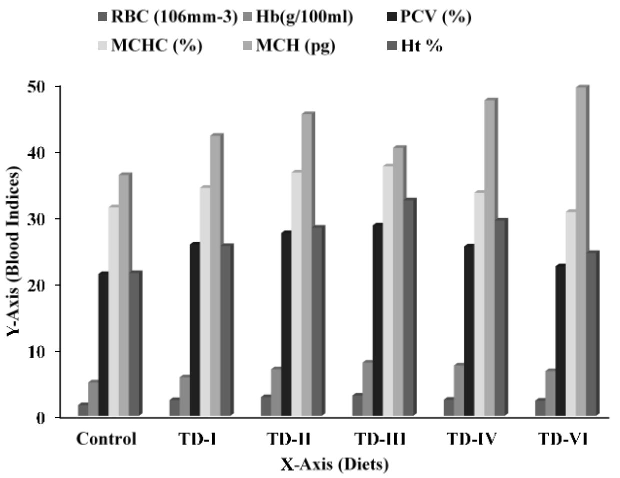

Fig. 1: The hematological indices of fingerlings (*C. mrigala*) fed on DCP supplemented PM based diet.

Minerals Absorption: Current study was done to evaluate the absorption of minerals fed on plant based diet supplemented with DCP. It was clear from table 1 that amount of all minerals calcium (Ca), sodium (Na),

Table 2: Analysis of haematological composition (%) of *C. mrigala* fingerlings fed on plant-based test diets supplemented with graded levels of Di calcium phosphate.

Diet	Level of DCP gkg^{-1}	RBC ($10^6 mm^{-3}$)	PLT	Hb(g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)	Ht %
0	0	$1.61±0.13^c$	$53.49±0.93^e$	$5.03±0.22^d$	$21.36±0.79^c$	$31.42±0.81^c$	$36.28±0.98^f$	$96.39±0.86^f$	$21.51±0.89^d$
1	0.20	$2.37±0.22^b$	$60.58±0.75^b$	$5.80±0.21_c$	$25.81±0.97^b$	$34.35±0.87^b$	$42.20±0.88^d$	$103.40±0.87^e$	$25.59±0.86^c$
2	0.40	$2.81±0.21^a$	$63.40±0.91^a$	$7.01±0.34^b$	$27.54±0.81^a$	$36.66±0.92^a$	$45.46±0.96^c$	$115.41±0.76^d$	$28.35±0.84^b$
3	0.60	$3.05±0.24^a$	$62.52±0.94^a$	$8.01±0.42^a$	$28.69±0.88^a$	$37.59±0.89^a$	$40.38±0.81^e$	$127.31±0.96^c$	$32.47±0.90^a$
4	0.80	$2.42±0.16^b$	$58.71±0.90^c$	7.58±0.22a	$25.52±0.89^b$	$33.60±0.89^b$	$47.54±0.85^b$	$158.68±0.78^b$	$29.43±0.80^b$
5	1.00	$2.28±0.26^b$	$56.42±0.75^d$	$6.73±0.33^b$	$22.53±0.98^c$	$30.72±0.99^c$	$49.48±0.91^a$	$160.46±0.71^a$	$24.53±0.86^c$
SE		.11761	.85213	.25324	.65028	.63289	1.09684	6.06657	.87902
Combined		.000	.000	.000	.000	.000	.000	.000	.000
Linear		.003	.075	.000	.179	.291	.000	.000	.000
Quadrant		.000	.000	.000	.000	.000	.385	.000	.000

Means within columns with various superscripts (a-c) differ considerably at $p < 0.05$. Data are three replicates' mean (shows Standard Deviations). RBC (Red blood cells) PLT (Platelet Count) Hb (haemoglobin) PCV (Packed cell volume) MCHC (Mean corpuscular hemoglobin concentration) MCH (Mean Corpuscular Hemoglobin) MCV (Mean Corpuscular volume) Ht (Hematocrit) St. E- Standard Error, L= Linear, O Quadratic, C= Combined.

potassium (K), phosphorous (P), copper (Cu), aluminium (Al), zinc (Zn), iron (Fe), and magnesium (Mg) was significantly similar in control as well as test diets. However, variations were observed in mineral content in feces of mori fingerlings fed with DCP supplemented plant based diet. It was noticed that maximum discharge of minerals (Ca, K, P, Al, Zn) in feces was observed in control diet having no supplementation of DCP while lowest discharge was observed at test diet 3 as observed in table 3. Lowest discharge of minerals results in maximum absorption of minerals.

Table 4 explain mineral absorption in mori fingerlings fed with diet having DCP supplementation. The highest mineral absorption values of Ca (72.21%), Na (72.45%), K (66.55%), and P (72.47%) were noted in test diet 3 at 0.60% DCP inclusion level as presented in figure 2. While the lowest values of mineral absorption for Ca (53.32%), K (47.78%), P (55.26%) were observed at control diet and lowest value for Na (60.44%) was noticed at test diet 5 having 1% DCP level. However, the highest mineral absorption values of Cu (69.65%), Al (68.48%), and Zn (63.36%) were recorded at the test diet 4, (0.80 %) DCP level and their lowest values (Cu: 56.42%, Al: 57.57% and Zn: 52.74%) were observed at control diet having no supplementation of DCP. The highest value of Fe (64.48%) was noted at test diet 2 having 0.40 % of DCP and the maximum value of Mg (51.51%) was noted at test diet 1 at 0.20 % of DCP while the lowest values for Fe (45.46) and Mg (39.89%) were notices at test diet 5 having 1% inclusion of DCP.

The study looks at the characteristics of *C. mrigala* fingerlings supplemented with a DCP diet in Tables 1, 3, and 4, which include calcium (Ca), sodium (Na), potassium (K), phosphorous (P), copper (Cu), aluminium (Al), zinc (Zn), iron (Fe), and magnesium (Mg). The graphical presentation of mineral absorption parameters was shown in figure 2.

The highest mineral absorption values of Ca (72.21%), Na (72.45%), K (66.55%), and P (72.47%) were noted in test diet 3 at the 0.60% DCP level. Whereas the highest mineral absorption values of Cu (69.65%), Al (68.48%), and Zn (63.36%) were recorded at the test diet 4 (0.80 %) DCP level. On the other hand, the highest value of Fe (64.48%) was noted at test diet 2 (0.40 %) level of DCP. However, the maximum value of mg (51.51%) was noted at test diet 1 (0.20 %) level of DCP. On the other hand, the lowest values of mineral absorption for Ca (53.32%), K (47.78%), P (55.26%), Cu (56.42%), Al (57.57%), and Zn (52.74%) were seen at the control diet (0 %) level of DCP. However, the lowest values of mineral absorption of Na (60.44%), Fe (45.46%), and Mg (39.89%) were noted at test diet 5 (1.00 %) level of DCP. In combined, linear and quadratic tests the values of all minerals (Ca, Na, K, P, Fe, Cu, Mg, Al, Zn) were significantly different.

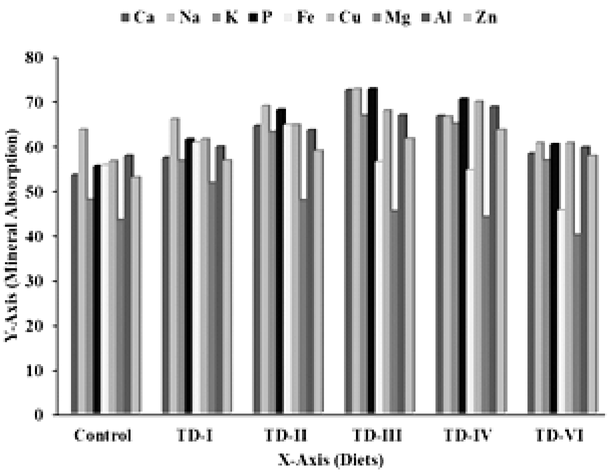

Fig 2: The MineralAbsorption of fingerlings (*C. mrigala*) fed on DCP supplemented PM based diet.

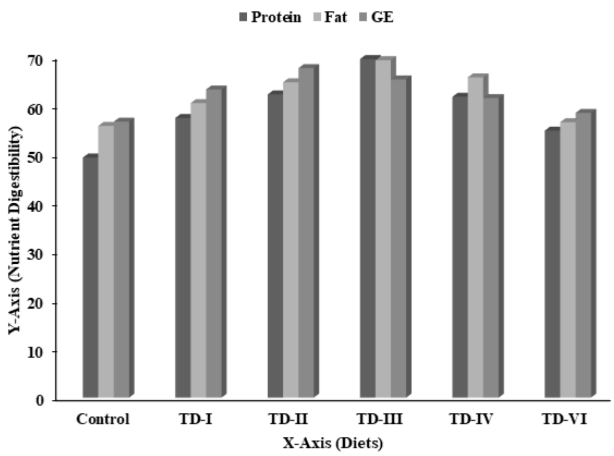

Fig 3: The nutrient digestibility of fingerlings (*C. mrigala*) fed on DCP supplemented PM based diet.

Nutrient digestibility: The study examines the nutrient crude protein (CP), crude fat (EE) and gross energy (GE) composition in diet, feces and their digestibility of in C. mrigala fingerlings supplemented with a DCP diet shown in tables 1, 5 and 6, respectively. Amount of nutrients in

Table 3: Analysis of minerals composition (%) in feces of *C. mrigala* fingerlings fed on plant-based test diets supplemented with graded levels of Di calcium phosphate

Diet	Level of DCP%	Ca	Na	K	P	Fe	Cu	Mg	Al	Zn
0	0	0.36±0.04$_{a}$	0.01±0.02^{ab}	0.74±0.04^{a}	0.93±0.05^{a}	0.05±0.09^{b}	0.02±0.04^{a}	0.01±0.03^{ab}	0.018±0.003^{a}	0.8±0.3^{a}
1	0.20	0.34±0.02^{ab}	0.09±0.02^{ab}	0.63±0.04^{b}	0.82±0.06^{b}	0.05±0.06^{b}	0.02±0.04^{ab}	0.05±0.04^{b}	0.017±0.001^{a}	0.07±0.03^{a}
2	0.40	0.29±0.04^{bc}	0.08±0.01^{ab}	0.54±0.01^{c}	0.68±0.05^{c}	0.05±0.04^{b}	0.19±0.04^{ab}	0.07±0.04^{ab}	0.015±0.003^{ab}	0.07±0.03^{a}
3	0.60	0.23±0.02^{c}	0.07±0.07^{b}	0.48±0.04^{c}	0.58±0.04^{d}	0.06±0.0026^{b}	0.07±0.02^{ab}	0.05±0.06^{ab}	0.014±0.002^{ab}	0.06±0.04^{a}
4	0.80	0.26±0.05^{c}	0.09±0.02^{ab}	0.49±0.04^{c}	0.61±0.02c^{d}	0.061±0.08^{b}	0.05±0.02^{b}	0.01±0.04$_{ab}$	0.012±0.02^{b}	0.05±0.03^{a}
5	1.00	0.33±0.04^{ab}	0.01±0.07^{a}	0.62±0.02^{b}	0.83±0.02^{b}	0.03±0.02^{a}	0.02±0.02^{ab}	0.04±0.05^{a}	0.07±0.02^{a}	0.017±0.02^{a}
SE		.0140812	.0004460	.0229621	.0316681	.0010808	.0000837	.0001150	.0000065	.0006220
Combined		.005	.176	.000	.000	.010	.128	.215	.076	.864
Linear		.045	.925	.000	.000	.005	.085	.122	.070	.342
Quadrant		.001	.020	.000	.000	.008	.053	.093	.052	.468

Means within columns with various superscript (a and b) differ considerably at P<0.05. Data are three replicates' mean (± shows Standard Deviations). Ca (calcium) Na (sodium) K (neo-Latin kalium) P(phosphorus) Fe (Iron) Cu (Copper) Mg (Magnesium) Al (Aluminium) Zn (zinc) St. E- Standard Error, L= Linear, O Quadratic, C= Combined.

Table 4: Analysis of minerals digestibility (%) of *C. mrigala* fingerlings fed on plant-based test diets supplemented with graded levels of Di calcium phosphate

Experimental Diet	Level of DCP%	Ca	Na	K	P	Fe	Cu	Mg	Al	Zn
0	0	53.32±0.62^{e}	63.40±0.83^{d}	47.78±0.81^{e}	55.26±0.86^{e}	55.51±0.76^{cd}	56.42±0.89^{e}	43.23±0.91^{d}	57.57±0.92^{e}	52.74±0.97^{e}
1	0.20	57.15±0.96^{d}	65.74±0.83^{c}	56.46±0.77^{d}	61.24±0.95^{d}	60.55±0.81^{b}	61.29±0.79^{d}	51.51±0.96^{a}	59.61±0.71^{d}	56.53±0.93^{d}
2	0.40	64.25±0.93^{c}	68.69±0.99^{b}	62.84±0.97^{c}	67.83±0.89^{c}	64.48±0.98$_{a}$	64.45±0.87^{c}	47.62±0.92^{b}	63.29±0.89^{c}	58.67±0.90^{c}
3	0.60	72.21±0.99^{a}	72.45±0.72^{a}	66.55±0.94^{a}	72.47±0.54^{a}	56.28±0.86^{c}	67.66±0.91^{b}	45.19±0.97^{c}	66.63±0.79^{b}	61.37±0.89^{b}
4	0.80	66.44±0.61^{b}	66.30±0.95^{c}	64.74±0.99^{b}	70.22±0.94^{b}	54.45±0.81^{d}	69.65±0.68^{a}	43.90±0.91^{cd}	68.48±0.90^{a}	63.36±0.70^{a}
5	1.00	58.07±0.98^{d}	60.44±0.87^{e}	56.55±0.83^{d}	60.13±0.98^{d}	45.46±0.58^{e}	60.43±0.92^{d}	39.89±0.96^{e}	59.47±0.00^{d}	57.55±0.74^{cd}
SE		1.5540914	.9369037	1.5526509	1.4866109	1.4315448	1.0988926	.9014289	.9812393	.8442327
Combined		.000	.000	.000	.000	.000	.000	.000	.000	.000
Linear		.000	.046	.000	.000	.000	.000	.000	.000	.000
Quadrant		.000	.000	.000	.000	.000	.000	.000	.000	.000

Means within columns with various superscripts (a - e) differ considerably at P<0.05Data are three replicates' mean (shows Standard Deviations). Ca (calcium) Na (sodium) K (neo-Latin kalium) P(phosphorus) Fe (Iron) Cu (Copper) Mg (Magnesium) Al (Aluminium) Zn (zinc) St. E- Standard Error, L= Linear, O Quadratic, C= Combined.

Table 5: Analysis of nutrient digestibility (%) in feces of *C. mrigala* fingerlings fed on plant-based test diets supplemented with graded levels of Di calcium phosphate.

Diet	Levels of DCP %	protein	Fat	GE
0	0	16.52±0.44^{a}	3.34±0.15^{a}	1.59±0.08^{a}
1	0.20	14.05±0.19^{c}	3.05±0.20^{b}	1.38±0.14b^{cd}
2	0.40	12.50±0.27^{d}	2.72±0.10^{c}	1.20±0.08^{d}
3	0.60	10.06±0.25^{e}	2.36±0.24^{d}	1.28±0.12^{cd}
4	0.80	12.34±0.31^{d}	2.57±0.08^{cd}	1.40±0.13^{abc}
5	1.00	14.73±0.49^{b}	3.29±0.11^{ab}	1.52±0.06^{ab}
SE		.0383504	.0943017	1.5505719
Combined		.006	.000	.000
Linear		.696	.020	.000

Means within columns with various superscripts (a-e) differ considerably at P<0.05. Data are three replicates' mean (shows Standard Deviations). P(protein) F(Fat) GE (Gross Energy). E- Standard Error, L= Linear, O Quadratic, C= Combined.

Table 6: Analysis of nutrient digestibility (%) of *C. mrigala* fingerlings fed on plant-based test diets supplemented with graded levels of Di calcium phosphate.

Diet	Levels of DCP %	protein	Fat	Gross Energy
0	0	49.29±0.90^{e}	55.78±0.92^{d}	56.64±0.81^{f}
1	0.20	57.41±0.83^{c}	60.46±0.91^{c}	63.24±0.87^{c}
2	0.40	62.23±0.79^{b}	64.72±0.92^{b}	67.64±0.67^{a}
3	0.60	69.50±0.69^{a}	69.26±0.76^{a}	65.31±0.86^{b}
4	0.80	61.76±0.97^{b}	65.73±0.85^{b}	61.48±0.89^{d}
5	1.00	54.81±0.71^{d}	56.54±0.95^{d}	58.43±0.87^{e}
SE		1.5505719	1.2050346	.9322564
Combined		.000	.000	.000
Linear		.000	.000	.742

Means within columns with various superscripts (a-f) differ considerably at P<0.05. Data are three replicates' mean (shows Standard Deviations). P(protein) F(Fat) GE (Gross Energy). E- Standard Error, L= Linear, O Quadratic, C= Combined.

all test diets and control diet were significantly similar. Maximum release of nutrients CP (16.52), EE (3.34) and GE (1.59) in feces was observed when fingerlings were fed with control diet having no supplementation of DCP while lowest discharge of CP (10.06) and EE (2.36) were observed at test diet 3 and lowest GE (1.20) values were noticed at test diet 2.

Highest digestibility values of CP (69.50%) and EE (69.26%) were noticed in test diet 3 at 0.60% inclusion of DCP while maximum GE (67.64%) values were noticed at test diet 2 as presented in figure 3. The second highest values of CP (62.23%) were observed at test diet 2 while second maximum EE (65.73%) and GE (65.31%) values were observed at test diet 4 and 3 respectively. These values differ significantly from other values. Whereas the lowest value of CP (49.29%), CF (55.78%) and GE (56.64%) were observed in control diet having no supplementation of DCP. In combined and linear tests, the values of protein and fat were significantly different while values of gross energy are non- significant.

DISCUSSION

The current study's findings indicate that the blood of fingerlings fed a diet devoid of DCP (0%) had the lowest levels of a few hematological indices, including Hb, RBCs, PCV, PLT, MCH, MCV, and Ht. Di Calcium Phosphate supplementation had a favorable impact on the fingerlings' blood profile. In this study, *C. mrigala* was selected as test animal that is known as one of the most important and valuable fish species in Pakistan (Chatha *et al*., 2023). Test diet 3 (0.60 g/kg) had the highest number of certain (RBC, Hb, PCV, MCHC and Ht) hematological indicators, in the fingerlings fed on DCP supplemented plant meal-based diet. MCH and MCV were highest at the test diet-5. Some similar outcomes to the current findings were discovered by Hussain *et al.* (2017) They used DCP-supplemented MOSM to analyze the hematological indices of *C. catla*, and the results showed that fish fed at the 0.50% level of DCP had better hematological values for RBCs, WBCs, and Hb, indicating improved physiological condition. Lazado *et al.* (2010) discovered that the Atlantic cod diet supplemented with DCP resulted in greater levels of RBCs in the blood is observed at 0.60 level of DCP. These results agreed with current work. Slightly different outcomes to the current findings discovered by Baruah *et al.* (2009). They found that combining 0.80% DCP with citric acid improved the hematological release of *rohita*. Sardar *et al.* (2007) discovered that the increase in WBCS was suitable at a DCP level of 0.75%. Our findings different slightly from this study.

The current study indicates that the highest mineral absorption values of Ca, Na, K, and P were noted in test diet 3 at the 0.60 g/kg DCP level. The results of Akpoilih *et al.* (2017) show that the maximal absorption of P, Ca, and K minerals in vundu catfish was seen at 0.70% DCP supplementation and decreased when its concentration rises to 1% of DCP. These findings are similar to current findings. Similar to the current study, Lemos *et al.* (2021) found the higher minerals deposition with better performance in aquatic animals when they were fed with MCP and DCP supplemented plant meal based diets.

The results of Cheng *et al.* (2016) likewise run counter to ours; they discovered that feeding yellow catfish plant proteins reduced the fishes' levels of phosphorous minerals. This is likely due to the phosphorus's restricted bioavailability in plant proteins. Contrary to current finding, it was reported by Javid *et al.* (2021) maximum minerals (P, Ca, Mg, Na) absorption was noticed when rohu fingerlings were fed with 10g/kg DCP level. Welker *et al.* (2016) reported that the presence of anti-nutritional components in the plant protein utilized caused zinc shortage in fish (*rainbow trout*) by rendering specific micronutrients, such as zinc, unavailable to the fish. According to Kaushik *et al.* (2018), Increased phosphorus loss was observed in *European sea bass* fed plant protein-based diets supplemented with DCP. Lund *et al.* (2011) reported similar outcomes in trout, proving that fish's phosphorus content was decreased by a large dose of plant proteins. Numerous studies carried out on animals have unequivocally demonstrated that the bioavailability of minerals from various feed items and supplements has a substantial impact on the amount needed for those minerals (Lall and Kaushik, 2021).

Slightly different outcomes to current findings Studies by Hardy and Lee (2010) found that when fish were fed a plant protein diet, the DCP of fish may be added to a plant-based diet to serve as a source of calcium and phosphorus. The bioavailability of minerals was unaffected. According to Suloma *et al.* (2013), *O. niloticus* was able to better use phosphorus when 80% of DCP was added to diets based on plant proteins. Some studies' findings ran counter to the findings of the current investigation. Hassan *et al.* (2013) found that the greatest ratio of triglycerides in *Nile tilapia* was at the 1 and 1.20% level of DCP, which were relatively different results. According to Liu *et al.* (2013), the serum of grass carp had higher concentrations of minerals (Ca, P, Zn, Mg) at several DCP levels, including 1.30, 1.40 and 1.50% levels of DCP, respectively. These findings are totally different from our work.

The study's findings showed that a plant-based diet with DCP supplements significantly increases the digestion of nutrients. Maximum gross energy was recorded at test diet 2, whereas maximum digestibility of nutrients (CP, EE) was recorded at diet 3 (0.60g/kg) level of DCP. Some findings similar to our work reported by Barzegar *et al.* (2014). Similar to recent research work, it was observed by Hien *et al.* (2023) that fish fed with diet having low levels of phosphorus results in low protein digestibility. They shared results that matched ours, demonstrating that *R rutilus* fed test diet 3 supplemented with 0.70% DCP had the highest digestibility of fat and protein. El-Komy and Shehab El-Din (2014) discovered that *Nile tilapia* fed on a plant meal-based diet supplemented with 1% DCP had the highest protein digestibility, which is quite different from our results. The endocrine system's regulation of fish homeostasis, biological availability from food, and P levels all have an impact on the absorption and metabolism of calcium (Lall, 2022).

Totally different outcomes to current findings reported by Hussain *et al.* (2018), they observed that fish fed a meal supplemented with 2.00 levels of DCP. Fish had a greater digestibility of protein and dry matter, these findings are different from our work. Different results were observed by Javid *et al.* (2021) who explained that adding varying DCP levels in diet of *L. rohita* does not affect nutrient digestibility of fish; all values were significantly similar to each other. For essential physiological and biochemical activities as well as to maintain their regular life processes, all aquatic species need minerals (Lall and Kaushik, 2021). The impact of alternative plant protein sources on the nutrient digestibility of various fish species was discovered by several research. Certain findings conflicted with the current findings. The differences in the results may be caused by the fish species' defensive mechanism, the source of the feed ingredient, the various methods used to process it, the way the feces are collected, and the viability of DCP.

Conclusions: These findings, together with the debate followed, pointed to the conclusion that the addition of DCP supplementation had a major impact on mineral absorption, nutrient digestibility and hematological indicators. Results indicated that fingerlings fed a plant-based diet at 0.60g/kg replacement level of DCP had a higher degree of nutritional digestibility and mineral absorption than the control group, which was fed a diet at 0g/kg replacement level of DCP. The results showed that in *C. mrigala* fingerlings, plant-based diet at 0.60g/kg replacement level of DCP improves the values of hematological indices (RBC, MCHC, Hb, Ht, PLT), mineral absorption (Ca, Na, K, P) and nutrient digestibility (CP, CF); as this level of DCP meet all the basic requirements for better development of fish (mori). After this level, feed absorption in body decreases on higher levels that shows that this level fulfils all the requirements of fingerlings. As per the given cost of ingredients, it was also concluded that the prepared diet was economical, cost effective and ecofriendly as compared to others having fish meal instead of sesame meal. The concept's potential to help the government create plans to expand and grow the aquaculture industry may present a range of options for people all over the world to support their way of life.

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Authors contribution: MS prepared the article and carried out the feeding trial. MMS organized, oversaw, and supplied all study materials. SMD helped in preparing the manuscript. FY and UE contributed to the writing. MB helped to the manuscript's rewriting.

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