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Improvement in Performance of Mori (*Cirrhinus mrigala*) Fingerlings Fed with DCP Supplemented Plant Meal-Based Diet

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MB conducted the feeding trial and prepared manuscript. MMS planned, supervised and provided all materials for research. SMD helped in manuscript preparing. FL and FY helped in writing, review, and editing. MS helped in editing and reshaping the manuscript.

Key words
Growth performance, Immunological indices, Carcass composition of *C. mrigala*, DCP

ABSTRACT

Present experimental work was conducted to examine the effects of a plant-based diet with supplemented di-calcium phosphate (DCP) on the growth performance, immunological indices, and carcass composition of *C. mrigala* fingerling. Six test diets 0, 1, 2, 3, 4, 5 containing varying level of DCP (0, 0.20, 0.40, 0.60, 0.80, 1 g/kg) were composed. Twenty fingerlings were stocked in each trial tank. Fish were given their basal diet two time in 24 h for 70 days with varied amounts of DCP plant-based food. Daily feces samples were taken from each tank and kept for chemical examination. The current study indicated that 0.60 g/kg of DCP supplementation in plant meal-based diet showed the highest weight gain (20g), final weight (27g), weight gain percentage (286%), and specific growth rate (1.50g). Fish fed a 0.60 g/kg amount of supplemented DCP plant-based diet had carcass compositions of protein (18.95), fat (8.94), and gross energy (2.05). In juveniles fed control diet (0g/kg), the lowest values of growth, immunological indices, and carcass composition were noted. The findings indicated that diet 3 (0.6g/kg) had the most suitable DCP level for formulating reasonably priced and sustainably compatible feed for *C. mrigala* fingerlings since it lowers the release of minerals and nutrients into water bodies as it was the DCP level that produced *C. mrigala* fingerlings in an inexpensive and environmentally friendly behavior.

INTRODUCTION

Food security is the top priority for countries to combat hunger and improve living standards, but persistent population growth in developing nations increases resource demands and challenges for sustainable development (Pradeepkiran, 2019). Worldwide population is rapidly growing at rate of 1.2 % per year, causing a need for a significant food supply to fulfill the growing need for food and proper nutrition in developing nations by 2050 (Godfray *et al*., 2010). World population's rapid rise has also led to an increase in food consumption (Yasmine *et al*., 2023). Aquaculture is vital for sustainable nutrition and protein supply (Rahman, 2015). Global food insecurity is a result of multiple international factors, chief within them being population growth, rising food costs, and hunger (Daviglus *et al*., 2002).

Fish meat provides energy from 14-143 kcal/g, and the aquaculture sector supplies food needs with high-quality protein, fatty acids, and essential nutrients (Abidi and Khan, 2010). The aquaculture industry is anticipated to significantly contribute to global food and nutrition supply, promoting sustainable development through minimal carbon footprint plant-based meals (Agbebi *et al*., 2009). Due to lower discharge of ingredients from plant meal based diet with addition of enzyme for phosphorus utilization, improvement in fish health and lower water pollution was observed from cost effective plant meal based diet (Sarfraz *et al*., 2020).

Aquaculture is a rapidly expanding sector of the food production industry that is replacing other food sources for humans that are high in protein (Sofia and Teresa, 2019). Aquaculture, the fastest-growing food industry globally, 31.1% increase in global fish production from 2004 to 2014, indicating significant potential for growth (Chen *et al*., 2022).

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Pakistan consumes 1.9 kg of fish, a high-quality, affordable protein source, providing health benefits like cardiac protection, neuroprotection, and anti-oxidation and anti-inflammatory properties (Tacon *et al.*, 2020). Around 3 billion people worldwide rely on fish and fish by-products for 20% of their protein needs, making them crucial in combating food poverty and malnutrition, particularly in Asian and African nations (Agbebi *et al.*, 2009).

Fish culture relies heavily on well-produced and planned feed, with feed expenses and feed effectiveness being key factors influencing the economy of fish farms (Wang *et al.*, 2020). Fish Meal, a protein-rich source in aquaculture, is highly sought after due to its exceptional growth performance (Li *et al.*, 2016). Fish meal is a primary source of protein for omnivorous fish, because of its high price and less availability there is requirement for alternative protein sources like plant-based protein which, reduces aquaculture costs (Hardy, 2010). Canola meal and moringa are suggested as optional fish feed, while byproducts plant meal recommended due to their availability, affordability, and environmental sustainability (Shahzad *et al*., 2020).

Black sesame seeds, a popular oilseed crop, are rich in lignans, tocopherols, and phytosterols, with a protein and lipid content that enhances animal health and performance (Trairatapiwan *et al.*, 2018). Black sesame belongs to Asteraceae family that is an annual herb, is a widely used edible oil crop in China, India, Malaysia, and other countries (Wang *et al*., 2023). Balanced availability of phosphorus and calcium is crucial for optimal growth performance in fish bones, and plant-based protein sources should supplement with phosphorus to prevent deficiencies (Razzaq *et al*., 2023). Fish feed contains phosphorus and calcium, but bioavailability varies. Modern aquafeeds incorporate plant sources, including DCP for mineral balance (Zhang *et al*., 2023).

Cirrhinus mrigala (mori), a Pakistani carp species, is a key contributor to the growing aquaculture sector, consuming organic matter and vegetable waste for high-quality fish protein (Tacon and Metian, 2008). *C. mrigala*, a popular Indian carp, is widely consumed in Pakistan due to its high meat quality, flavor, and economic and commercial value in Pakistan's freshwater reservoirs. It has wide distribution in freshwater reservoirs of Pakistan of substantial economic importance and market value (Rauf, 2015). For carp, supplemental feed accounts for almost 50% of the total cost. Aquaculture will need to produce food in the future, thus economically viable, high-quality feeds will be essential (Yang *et al.*, 2012). Therefore, present study was planned to examine the efficiency of DCP inclusions on the growth and deposition of nutrients and immunology of mori fingerlings fed on plant by-products-based diet.

MATERIALS AND METHODS

Fish fingerlings with trial conditions

The study was carried out at the Fish Nutrition Lab of the Zoology Department at the University of Education Township Campus Lahore, Pakistan. *C. mrigala* were purchased from Manawa Fish Hatchery Lahore, Pakistan, with comparable lengths and average weights. The fingerlings were gradually introduced to acclimated to the experimental conditions over a period of two weeks. A plant meal-based diet was used to composed the experimental diet, with DCP acting as the test ingredient. For the stock of experimental fingerlings, specially made 70-liter aquaculture aquarium were utilized. The fish were fed a basic diet twice a day. Throughout the whole trial, an air pump was used to deliver O_2 through capillary system. The usual range of water quality measures, such as pH, dissolved oxygen, and temperature, were consistently monitored.

Table I. Ingredients composition (%) of experimental diets (TD).

Ingredients	Control	TD-1	TD-2	TD-3	TD-4	TD-5
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	9.8	9.6	9.4	9.2	9
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
Chromic oxide	1	1	1	1	1	1
DCP (g/kg)	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.

Experimental design

One hundred twenty mori fingerlings, with an average of 2 g, were used in the experiment. Six test diets (TD) with DCP ingredients of 0, 0.20, 0.40, 0.60, 0.80, 1g/kg were mixed until they had a uniform consistency. The diets were stored in sealed bags at room temperature until it was

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time to feed the fish (0,1, 2, 3, 4, 5) experimental tanks were set up. The temperature of the water stayed around 25 degrees. Feeding the fish a known that measuring the amount of excreted in the feces was yield the apparent digestibility coefficients for protein, crude fat, and crude fiber. A sample of the fish's muscle tissue were analyzed to ascertain its body composition.

Feed pellet formation

Each of the feed ingredient was purchased from the local market in Lahore. Standard procedures as applied by AOAC (1995), were used to assess the feed's chemical composition before the experimental diet was created. In a blender, the components were combined for five to ten minutes. Fish oil and distal water were added to the mixture progressively while it was being mixed. The resulting of mixture was taken to produce 2mm-diameter pellets. Subsequently, the pellets were dried in a drying chamber at 40 °C until the moisture level reached approximately 10%. The pellets were dried, then ground into the necessary particle sizes and stored at 4°C.

Feeding procedure and sample collection

During the trial, the diets were provided to each of the 6 groups of *C. mrigala* fingerlings twice a day for two h in the morning and the evening. The waste was carefully collected, taking special care to avoid breaking the feces. The faeces were collected, air-dried at 65 °C inside an oven for 3 to 4 h, and then stored for chemical analysis.

Growth performance

Juveniles in each tank were weighed at the beginning and end of the trial to analyze fingerling growth parameters using the following standard formulae:

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

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

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

Immunological indices

Blood samples were obtained for immunological parameter without the use of anticoagulants. Using blood samples, the numbers of leukocytes and erythrocytes were ascertained. The counts for neutrophils, monocytes, eosinophils, and lymphocytes were ascertained using the differential counting method. Samples were separated using the centrifugation process and frozen at -20 °C until analysis.

$$\text{MCHC} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

$$\text{MCV} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{MCH} = \frac{\text{HB}}{\text{RBC}} \times 10$$

Carcass composition

From each replicate, three fish were chosen at random, and they were killed according to practices so that the carcass composition could be examined (AOAC, 1995). Gross energy across the entire body was measured using an oxygen bomb calorimeter. The crude protein was examined using the Micro Kjeldahl apparatus. The sample was oven dried at 105°C for 12 h in order to ascertain the moisture content of the carcass. The soxhlet apparatus was utilized to extract crude fat utilizing the petroleum ether extraction method, while digestion using 1.25% H_2SO_4 and 1.25% NaOH as a loss on ignition of dried lipid-free residues was used to identify crude fiber. Ash was measured in an electric furnace (Eyela-TMF 3100) at 650°C for 12 h at a steady temperature. The following formula was used to get the total amount of carbohydrates:

$$\text{Total carbs (\%)} = (100 - \text{moisture} + \text{protein\%} + \text{fat \%} + \text{crude fiber\%} + \text{Ash})$$

Statistical analysis

Finally, data of growth performance, immunological indices and body composition of fish were subjected to analysis of variance (one-way) by using the SPSS and analyzing by Dukan's test at significance level of P<0.05.

RESULTS

Table II show results of growth parameters of mori juveniles was impacted by varying levels of DCP supplemented plant meal-based diet and presented in Figure 1. The test diet 3 (0.60 g/kg) showed the greatest improvement in the growth, FW (27.55g), WG (20.40g), and WG % (286.43%). Lowest FW (21.42g), WG (14.30g) and WG % (201.14%) were found in juveniles fed with control diet (0 g/kg) level of DCP. Fingerlings fed control diet (0 g/kg) level of DCP supplemented diet showed poor FCR (1.78) value. Best FCR (1.25) was recorded in fingerlings that were fed at diet 3 (0.60 g/kg) level of DCP. *C. mrigala* fingerlings fed on diet 3 (0.60 g/kg) supplementation of DCP showed best SGR (1.50). When fingerlings were fed at control diet (0 g/kg) level of DCP, poor SGR (1.22) was observed. Survival rate values of all fingerlings were non-significant. In combined and quadratic tests values of FW, WG, WG%, FCR and SGR in combined and quadratic test values are significantly different while in linear test all values are non-significant.

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Table II. Growth parameters of mori fingerlings fed a diet based on plant meal supplemented with dicalcuim phospahte (DCP).

Test diets	DCP (gkg^{-1})	IW (g)	FW (g)	WG (g)	WG (%)	W gain/70 (g)	Feed in-take (g)	FCR	Survival rate	SGR
0	0	7.12±0.44^{a}	21.42±0.92^{d}	14.30±0.68^{d}	201.14±13.44^{c}	0.20±0.01^{d}	0.36±0.03^{a}	1.78±0.10^{a}	91.49±3.05^{a}	1.22±0.05^{c}
TD-1	0.2	7.14±0.37^{a}	22.45±0.95^{cd}	15.30±0.86^{cd}	214.61±15.94^{ab}	0.22±0.01^{cd}	0.36±0.02^{a}	1.64±0.01^{b}	91.21±3.11^{a}	1.27±0.06^{b}
TD-2	0.4	7.11±0.28^{a}	25.51±0.95^{b}	18.40±0.67^{b}	258.64±1.56^{a}	0.26±0.01^{b}	0.36±0.02^{a}	1.39±0.03^{c}	96.58±2.97^{a}	1.42±0.00^{a}
TD-3	0.6	7.15±0.47^{a}	27.55±0.87^{a}	20.40±1.10^{a}	286.43±32.08^{a}	0.29±1.10^{a}	0.36±0.02^{a}	1.25±0.05^{d}	96.48±3.06^{a}	1.50±0.09^{a}
TD-4	0.8	7.13±0.37^{a}	23.45±0.87^{c}	16.32±0.66^{c}	229.05±0.87^{ab}	0.23±0.01^{c}	0.34±0.01^{a}	1.44±0.11^{c}	96.58±2.97^{a}	1.32±0.04b^{c}
TD-5	1	7.14±0.40^{a}	21.66±0.72^{d}	14.52±1.11^{d}	204.37±26.34^{c}	0.21±0.02^{d}	0.34±0.03^{a}	1.65±0.09^{ab}	91.40±2.90^{a}	1.23±0.10^{c}
SE		0.077	0.561	0.560	8.415	0.008	0.004	0.046	0.865	0.027
Combined		1.000	0.000	0.000	0.001	0.000	0.346	0.000	0.076	0.001
L		0.962	0.168	0.167	0.373	0.167	0.084	0.002	0.305	0.384
Q		0.990	0.000	0.000	0.000	0.000	0.451	0.000	0.014	0.000

Means within columns with various superscripts (*a-d*) differ considerably at $P < 0.05$. Data are three replicates mean (shows standard deviations). CM, copra; IW, initial weight; WG, weight gain; FW, final weight; FCR, feed conversion ratio; SGR, specific growth rate; St. E, standard error; L, linear, Q, quadratic; C, combined.

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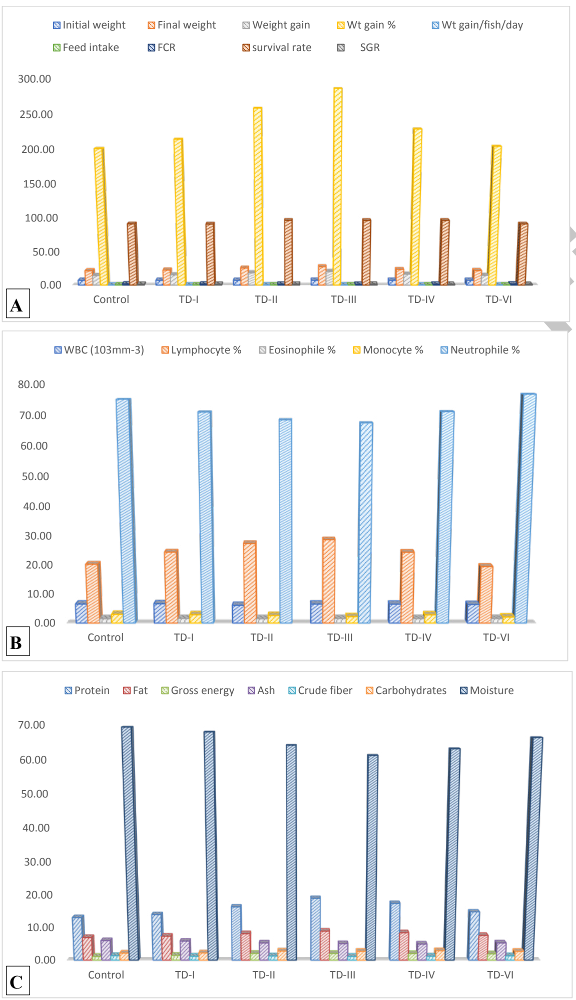

Fig. 1. Effect of DCP added sesame meal on growth rate (A), immunological indices (B) and carcass composition (C) of fingerlings of *Cirrhinus mrigala*.

Table III explained results of immunological indices included WBC (103mm^{-3}), lymphocytes, monocytes, eosinophil and neutrophils of *C. mrigala* fingerlings fed on DCP supplemented plant meal-based diet. Fish given TD^{-1} (0.20 g/kg of DCP) had the greatest percentage of WBC (6.73) and eosinophiles (1.56%) as explained in Figure 1B. In contrast lowest count of WBC (6.19%) was found in juveniles fed on test diet 2 (0.40 g/kg of DCP) and the lowest value of eiosinophile were found in the fish that were fed on test control diet (1.48) level of DCP. The fish that were fed TD-3 with (0.60 g/kg) of DCP supplementation had the greatest lymphocyte percentage (28.70%). The lowest count of lymphocyte (19.54%) was found in fish fed on TD-5(1 g/kg.) Fish fed 0 g/kg of DCP level had the greatest percentage of monocytes (3.02%). The lowest value of monocyte% (2.09%) was found in TD-5 (1 g/kg). The fish fed test diet 5 showed the greatest neutrophil count (76.85%) when 1.25 g/kg of DCP was administered. Whereas the lowest value of neutrophile (67.53%) was found in the fish that were fed on TD-3 (0.60 g/kg). In combined and quadratic tests, values of lymphocytes and neutrophile are significantly different while values of WBC, eosinophile and monocyte are non-significant in all three (combined, linear and quadratic) tests.

Table IV explained the body composition of mori juveniles fed on supplementation of DCP with plant meal-based diet. DCP supplemented diets significantly affected fish body composition as compared to control diets as explained in Figure 1C. The results demonstrated that adding DCP to the diet enhanced the body composition of *C. mrigala*. The fish given test diet 3 (0.60 g/kg) of DCP supplementations had the highest values of crude protein (18.95%), crude fat (8.94%) and the other hand fish given test diet 2 (0.40 g/kg) of DCP supplementations had the highest values of gross energy, according to the results.

Table III. Immunological indices of mori fingerlings fed a diet based on plant meal supplemented with DCP.

Test diets	DCP (gkg^{-1})	WBC (10^{3}mm^{-3})	Lymphocyte (%)	Eosinophils (%)	Monocyte (%)	Neutrophils (%)
0	0	6.63±0.37^{a}	20.33±0.75^{c}	1.48±0.19^{a}	3.02±0.20^{a}	75.17±0.76^{b}
TD-1	0.2	6.73±0.31^{a}	24.49±0.88$_{b}$	1.56±0.18^{a}	2.87±0.23^{a}	71.07±0.86^{c}
TD-2	0.4	6.19±0.20^{c}	27.39±0.85^{a}	1.49±0.31^{a}	2.58±0.19^{b}	68.54±0.69^{d}
TD-3	0.6	6.62±0.26^{a}	28.70±0.90^{a}	1.55±0.09^{a}	2.21±0.24^{bc}	67.53±1.08^{d}
TD-4	0.8	6.59±0.22^{b}	24.44±0.85^{b}	1.51±0.16^{a}	2.85±0.33^{a}	71.20±1.28^{c}
TD-5	1	6.47±0.26^{b}	19.54±0.80^{c}	1.52±0.19^{a}	2.09±0.20^{c}	76.85±0.72^{a}
SE		0.069	0.828	0.039	0.096	0.828
C		0.281	0.000	0.992	0.002	0.000
L		0.569	0.507	0.898	0.001	0.106
Q		0.518	0.000	0.838	0.620	0.000

For statistical details and abbreviations, see Table II.

Table IV. Carcass composition of mori fingerlings fed a diet based on plant meal supplemented with DCP.

Test diets	DCPgk g^{-1}	Protein (%)	Fat (%)	Gross energy (kcal kg^{-1})	Ash (%)	Crude fiber (%)	Carbohy-drates (%)	Moisture (%)
0 (Control)	0	13.07±0.43^{e}	6.98±0.37^{d}	1.12±0.24^{c}	6.00±0.19^{a}	1.37±0.11^{a}	2.15±0.36^{c}	69.30±0.82^{a}
TD-1	0.2	14.00±0.35^{de}	7.39±0.50^{d}	1.46±0.14^{c}	5.85±0.20^{a}	1.18±0.18^{a}	2.20±0.19^{c}	67.92±1.36^{ab}
TD-2	0.4	16.36±0.78^{c}	8.16±0.19^{c}	2.05±0.19^{a}	5.27±0.26^{b}	1.28±0.15^{a}	2.78±0.28^{a}	64.10±0.91^{c}
TD-3	0.6	18.95±0.42^{a}	8.94±0.39^{a}	2.05±0.10^{a}	5.05±0.29^{b}	1.14±0.18^{a}	2.69±0.33^{ab}	61.17±0.99^{d}
TD-4	0.8	17.42±0.81^{b}	8.47±0.43^{b}	2.02±0.18^{a}	4.93±0.20^{b}	1.20±0.12^{a}	2.89±0.23^{a}	63.08±1.25^{c}
TD-5	1	14.83±0.53^{d}	7.65±0.68^{c}	1.93±0.11^{b}	5.28±0.31^{b}	1.33±0.10^{a}	2.68±0.21^{b}	66.31±0.09^{b}
SE		0.503	0.184	0.92	0.108	0.034	0.087	0.708
Combined		0.000	0.002	0.000	0.001	0.360	0.025	0.000
L		0.000	0.006	0.000	0.000	0.654	0.004	0.000
Q		0.000	0.001	0.000	0.006	0.079	0.075	0.000

DCP, di calcium phosphate. For statistical details and abbreviations, see Table II.

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On the other hand, crude protein (13.07%), crude fat (6.98%), and gross energy (1.12 kcal/g), fish given control diet (0 g/kg of DCP) had the lowest values. Whereas it found that the highest value of ash (6.00%) in the fish that were fed on test control diet (0 g/kg). fingerlings fed on test-diet 3 (0.60 g/kg of DCP) had the lowest concentration of Ash (4.93%). The highest amount of crude fiber (1.37%) was observed in mori fed test control diet (0 g/kg of DCP supplementations), according to the results. The lowest value of crude fiber (1.14%) was found in TD 3 (0.60 g/kg) level of DCP. Maximum amount of carbohydrates (2.89%) was noted in fish given a diet supplemented with 4 (1 g/kg) level of DCP. The lowest value of carbohydrates (2.15%) was found in test control diet (0 g/kg) level of DCP. Fish given control diet (0 g/kg of DCP) had the highest moisture content (69.30%), while fish fed TD 3 (0.60 g/kg of DCP) had the lowest moisture content (61.17). In combined, linear and quadratic tests values of protein, Gross energy, fat, ash and Moisture are significantly different while crude fiber and carbohydrates values are non-significant.

DISCUSSION

Current research work was done to study the effects of DCP supplementation on fingerling's growth using varying amounts of DCP (0, 0.20, 0.40, 0.60, 0.80, 1 g/kg) added to a plant-based diet. Current research experiment revealed that the inclusion of DCP to the PM based diet considerably improves growth performance of the *C. mrigala* fingerlings. Fish given TD-3 (0.60 g/kg) that the highest weight increase (20.40g), weight gain percentage (286.43), and SGR (1.50) values when plant meal-based diets were supplemented with DCP. It was also determined that mori fingerling growth was inhibited by increased DCP supplementation doses when fed a plant meal diet. A diet high in plant meal was thought to be a healthy source of protein (Yusup and Nugroho, 2017). Similar to recent studies, Yusup and Nugroho (2017) examined the impact of supplementing DCP in *C. mrigala* diet to study growth performance and came to the conclusion that doing so at a rate of 0.60% improved *C. mrigala* growth and increased weight gain, weight gain percentage, and SGR values (Hepher and Sandbank, 1984). Numerous researchers have noted that adding a plant meal-based diet supplemented with DCP to fish diets at an inclusion rate of 0.79% can improve growth outcomes which is almost in line with current findings (Jauncey, 1998). Present study showed reduced growth performance at high inclusion levels of DCP supplementation with plant meal-based diet similar results were shown by different researchers such as rohu, tilapia and mori (Jackson *et al*., 1982; Hassan *et al*., 2022). Rana and Das (2016) also found similar results that greater replacement levels of DCP (0 and 0.25%) led to reduced weight increase, weight gain %, and higher FCR in the diets of *L. rohita* fingerlings. Apines *et al*. (2015) examined the effect of DCP inclusion on the growth performance of mori and reported that up to 1.25% of DCP can be utilized in fish diets. Olude *et al*. (2008) found rather different results when they analyzed the impact of supplementing DCP with a PM based diet on the growth of tilapia. They came to conclusion that DCP could be included to fish feed up to 0.5% without affecting fish performance. In contrast to recent studies, some researchers discovered that *C. mrigala* FCR values are unaffected by DCP supplementation at 0.9% on a plant meal-based diet (Yusup and Nugroho, 2017).

According to present work, the immunological indices of mori juveniles improved by addition of DCP in plant-based diet at varying inclusion levels and maximum and minimum levels of WBC (10^3 mm^{-3}), lymphocyte, monocyte, neutrophils and eosinophils were noticed. Mahboub and Tartor (2020) have shown that feeding Nile tilapia a diet having 0.35 g/kg of DCP had a variety of protective benefits on the immune system. *L. rohita* Ali *et al*. (2020) conversely observed a rise in mean WBCs, particularly in those fed 0.20% DCP, highlighting the significant impact of DCP supplementation on WBC levels. Silvia *et al*. (2024) further demonstrated that, in contrast to our findings, the biggest increase in WBC levels which include neutrophils, monocytes, and lymphocytes was observed in *O. niloticus* at a dosage of 1.20 g/kg of DCP.

Hassan *et al*. (2022) provided support for these findings by highlighting the rise in lymphocyte levels demonstrating a spectrum of protective benefits on the immune response in *Cyprinus carpio* following a 1 g/kg supplemented DCP-based diet. A study by Naylor *et al*. (2000) found that feeding *C. carpio* a diet based on DCP caused a drop in lymphocyte numbers, which is contrary to the current findings. There's still a lot to learn about fish immunology using DCP.

In the current investigation, at the control diet, monocyte values were raised with DCP conversely, Hidayat and Harpeni (2014) discovered that a diet containing supplemented DCP effectively boosted non-specific immune responses, notably monocytes. Similarly, adding DCP to fingerling's diet was found to significantly enhance number of monocytes (Yuli *et al*., 2021).

According to recent findings, supplementing DCP plant-based diet to fingerlings of *C. mrigala* at varying inclusion levels increased the carcass composition of the animals. The current study found that the highest levels of CP, EE and GE were observed at 0.60 k/kg DCP level, while the lowest levels were found at the 0 k/kg DCP level. The carcass composition of fish fed with PM based diet having DCP inclusion has not yet been studied. Khan *et al*. (2012) provided an explanation of the current finding, stating that in Indian major carps, the proportion of crude protein and fat in meals supplemented with DCP plant-based diets compared to control diets (without plant-based diets). Recent research indicates that the protein level at the initial inclusion level is higher than the protein level at the end (high) inclusion level. In contrast to the current findings Mukhopadhyay and Ray (1999) noted that *L. rohita* fingerlings fed with DCP supplementation at an inclusion level of 0.77% had a greater carcass composition in contrast to recent research. Tayyab *et al*. (2014) described the impact of adding plant-based supplements to the DCP diet (sunflower, rice polish), and their findings demonstrated that at high inclusion levels of PM diet, ash content of juveniles also rises. Current research effort detected a high crude fiber concentration of 0% and a low crude fiber content of 0.75% in the supplemented DCP plant-based diet (Sánchez-Muros *et al*., 2016) which shows that maximum levels of DCP leads to lower values of crude fiber. In present study, the maximum moisture values were found at 0 g/kg replacement and the lowest at 0.60 g/kg replacement. Other researchers concurred, suggesting that maximum protein and lipid levels in the fingerlings might be the reason of minimum moisture levels at low levels (Giri *et al*., 2000). DCP supplementation to replace fish meal to study carcass composition in *Nibea miichthioides* was used by Wang *et al*. (2006) who showed that increased moisture levels at maximum levels. This finding is similar to mine, which showed higher moisture values at higher inclusion levels. The differences in the results might be caused by the fish species' immune system, the source of

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the feed ingredient, the various methods used to prepare it, the process of feces are collected, and the sustainability of DCP (Hussain *et al*., 2017).

CONCLUSION

These results, together with the discussion, led to the conclusion that adding DCP supplementation plays a remarkable role in improving growth, immunological indices, and carcass composition. Results showed that supplementing DCP at diet 3 (0.60g/kg) is an effective way to raise the growth, immunological indices and body composition of *C. mrigala*.

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Statement of conflict of interest

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