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MARZIA BATOOL

Roll No. SU92-MSZGW-F22-003

FOS

Efficacy of Di-Calcium Phosphate (DCP) Supplemented Plant Meal-Based Diet on Growth Performance, Carcass Composition and Immunological Indices of *Cirrhinus mrigala* (Mori) Fingerlings



SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

In Partial Fulfillment of the

Requirement for the Degree of

MS Zoology

By

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This is to certify that the thesis entitled “**Efficacy of Di-Calcium Phosphate (DCP) Supplemented Plant Meal-Based Diet on Growth Performance, Carcass Composition and Immunological Indices of *Cirrhinus mrigala* (Mori) Fingerlings**” submitted by “**Marzia Batool**” has been accepted towards the partial fulfillment of the requirement for MS/M.Phil. “**MS Zoology**”. The quality of the work contained in this thesis is adequate for the award of degree.

Supervisor Name: Sadia Muhammad Din

Designation: Lecturer

Department of Biological Sciences

Signature: _____

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This is to certify that the research work presented in this thesis, titled “**Efficacy of Di-Calcium Phosphate (DCP) Supplemented Plant Meal-Based Diet on Growth Performance, Carcass Composition and Immunological Indices of Cirrhinus mrigala (Mori) Fingerlings**” was conducted by “**Marzia Batool**” under the supervision of “**Ms. Sadia Din**”

No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Science in the field of “**Zoology**” in Faculty of Sciences at The Superior University, Lahore.

Student Name:

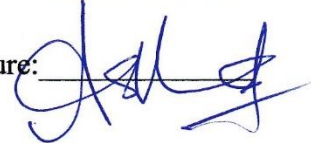
Signature: Marzia Batool

Examination Committee:

Session Chair: Dr. Noor Ul Ain

Signature: _____

a) External Examiner: Dr. Asma Ahmed

Signature: 

b) Internal Examiner:

Signature: _____

c) Supervisor Name: Ms. Sadia Din

Signature: _____

d) Name of HOD: Prof. Dr. Uqba Mehmood

Signature: _____

e) Name of Dean: Prof. Dr. Mohammad Naveed Babur Signature: _____

f) Controller Examination: Dr. Muhammad Haris

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Dedication

I dedicate this effort to:

My loving Father, **Murtaza Shah** whose inspiration towards knowledge served me as a beacon of light, he made me what I am today.

My loving Mother, **Naseem Akhtar**

Who taught me the first word to speak the first step to take And Who is universe for me whose hands are always raised for me and dedicated specially a personality under whose feet my heaven lies who support, help and pray for me in whole of life. Who always inspired and encouraged me, who always wish for my success and prosperity.

Acknowledgement

In the name of Allah, the most Gracious, the most Merciful.

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I would like to express my profound gratitude to my supervisor for his/her wise counsel and encouraging attitude towards this study. I am extremely grateful to him/her for immensely facilitating me during my study period by ensuring the provision of favorable circumstances and conducive environment. This project would not have been possible without his support and expert guidance.

In the end, I would like to extend my deepest gratitude to my family members. Without their encouragement, I would not have been able to complete this endeavor. (You can always choose your own wordings; this is only a format/sample to be followed for uniformity of all thesis).

Marzia Batool

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List of Abbreviations

DCP	Di-calcium phosphate
FM	Fish meal
PM	Plant meal
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
GE	Gross energy
CP	Crude protein
FCR	Feed conversion ratio
SGR	Specific growth rate
WG	Weight gain
IW	Initial weight
FW	Final weight
FI	Feed intake
SS	Sum of square
MS	Mean of square
CA	Crude ash
CF	Crude fiber
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
WBCs	White blood cells
Hb	Hemoglobin
PCV	Packed cell volume
P-value	Probability
ADC	Apparent digestibility coefficient
CRD	completely randomized design
NaOH	Sodium hydroxide
SPSS	Statistical Package Social Science
L	Linear
Q	Quadratic
C	Combined
D.F	Degree of Freedom
S. E	Standard error

ABSTRACT

Plant-based protein benefit fish nutrition, nutrient utilization, development, protein retention, digestibility, and bioavailability as well as their biochemical composition, resistance, and stress responses. Di-Calcium Phosphate is a cost-effective alternative to other sources of phosphorus, such as fishmeal. It promotes the growth of fish by providing essential minerals and phosphorus. The current study was to determine that a plant-based diet enhanced with Di-Calcium Phosphate affected the immunological indices, carcass composition, and growth performance of fingerlings *Cirrhinus mrigala*. Diet containing Di-Calcium Phosphate (DCP) are frequently utilized to promote the health and performance of animals. By using various concentration of DCP (0, 0.20, 0.40, 0.60, 0.80, 1 g/Kg) six test diets 0, 1, 2, 3, 4, 5 were prepared. In an aquarium, the experiment was carried out with 20 fingerlings stocked in each replicate. For 70 days, the six groups were fed twice a day on their individual diets supplemented with different amounts of DCP plant-based diet. Fecal samples were taken daily from every tank and kept for chemical analysis. The objective of this research is to investigate that the growth rate, carcass composition, and immunological indices of *C. mrigala* are enhanced by replacing a sesame and DCP mixture for fish meal. Growth rate, carcass composition, and immunological indices parameter were evaluated by one-way analysis of variance (ANOVA). The current study findings indicated that the maximum weight rise (20g), final weight (27g), weight gain percentage (286%), and specific growth rate (1.50g) were recorded when a plant meal-based diet supplemented with 0.60 g/Kg of DCP. Similarly, immunological indices parameters were improved at all test diets. Carcass composition protein (18.95%), fat content (8.94%), and gross energy (2.05%) were measured in fish given a DCP plant-based diet supplemented with 0.60 g/Kg. Fingerlings given a control diet (0 DCP) showed the lowest growth, immunological indices, and carcass composition values. Inclusion of DCP supplementation with plant meal-based diet mixture in feed results showed that test diet 3 (0.60g/Kg) is an effective way to raise the growth performance, immunological indices and carcass composition of *C. mrigala*.

Keywords: *Cirrhinus mrigala*, supplemented in DCP plant-based diet, Growth performance, Immunological indices and Carcass composition.

CHAPTER 1

INTRODUCTION

Ensuring food security is the top priority for all countries worldwide in order to combat hunger and raise economic standards of living. This problem persists globally in the state of developing nations today [1]. Consistent population growth increases further demand on already-strained resources and creates new difficulties for sustainable development [2]. If current growth rates continue, the world population would have grown to almost 9 billion people by 2050, with the current rate of increase being around 1.2 percent annually [3]. The population of developing nations is growing daily, which rapidly raising the demand for food and proper nutrition. A rising global population requires a significant food supply [4].

world population rapid rise has also led to an increase in food consumption [5]. Rapid population expansion has a greater impact on developing nations, which has resulted in a decrease in food supply in recent years. Our ability to produce food in balance will be harmed by increased competition for land, water, energy, and overfishing, as well as by the urgent need to reduce the negative environmental effects of food production [6]. Acknowledging the need to feed the continuously growing global human population and providing a nutritious protein meal in a sustainable manner for such a vast population, aquaculture plays a vital role in ensuring sufficient nutrition [7]. Global food insecurity is a result of multiple international factors, chief within them being population growth, rising food costs, and hunger [8].

The main source of energy for individuals living beside the coast is still vegetables, despite fishing is still vital. Fish meat has an energy content that ranges from 14–143 kcal/g [9]. The aquaculture sector to supply our food needs, using fish as our primary source of protein since it supplies high-quality protein, significant fatty acids, and other nutrients which are vital to human health [10]. While fish feeds over 2.9 billion people it is included in diets as a premium supply of protein and as a way to address the problem of food scarcity [11]. The fishing industry will contribute more to global food and nutrition supply in the years to come [12]. Aquaculture is expanding faster than other food-producing sectors. It enhances sustainable development by

encouraging the production of meals derived from animals that have minimum carbon footprints [13].

Aquaculture is a quickly developing food production industry that is quickly taking over as the primary source of high-protein food for humans [14]. Aquaculture accounts for 47% of all fish produced worldwide; by 2050, however, that percentage is expected to rise to roughly 52% [15]. Aquaculture, one of the most significant food industries globally, provides a new source of animal protein to the world's expanding population. [16]. Aquaculture is the food industry with the fastest rate of growth in the world. Global fish production from aquaculture was 31.1% in 2004, 42.1% in 2012, and 44.1% in 2014 [17].

The number of fish and seafood consumed varies by region in the world; The typical yearly intake per individual may vary from 1 kilogram to more than 100 Kg. [18]. Fish provide many health benefits such as cardiac protection, neuroprotection, and anti-oxidation and anti-inflammatory qualities. These qualities make fish a great source of high-quality protein [19]. Around 3 billion people worldwide rely on fish and fish by-products to provide 20% of their daily needs for animal protein [20]. Since fish is an affordable and easily accessible source of animal protein for nutritional needs, especially in remote areas, it is a vital weapon in the battle against food poverty and malnutrition. It has the necessary amounts of protein, vitamins, and other nutrients for human food [21]. More than 30% of all animal protein comes from fish products, which are the main source of protein in the majority of Asian and African nations [22].

Fish have numerous health advantages, such as cardiac protection, neuroprotection, anti-oxidation, and anti-inflammatory properties. Because of these characteristics, fish is a great source of high-quality protein [23]. The high protein content of fish feed, which is mostly derived from expensive and in-demand fish meal, has raised feed production cost [24]. Fish culture heavily relies on properly produced and precisely planned feed. The main variables governing the fish farm economy are feed costs and feed efficiency [25].

One of the most preferred protein sources for aquaculture diets is fish meal, because it has the best development performance in fish because of its remarkable nutritional features, which include a significant amount of protein, balanced quantities of fatty acids and amino acids, and high digestibility [26]. For carnivorous fish, fish meal (FM)

has long been a significant source of protein. The alternative protein sources were pushed by rising demands, declining accessibility, and an increase in the price (about 220 PKR/Kg) of FM. About 50–60% of all costs associated with aquaculture output go toward fish feed [27]. It has been shown in numerous research studies that replacing FM with plant-based protein sources is beneficial when done so in certain nutritionally balanced situations [28]. In the aqua feed used for carnivorous fish, which frequently have greater dietary protein requirements, fish meal (FM) has been a significant source of protein [29].

plant-based proteins benefit fish nutrition, nutrient utilization, development, protein retention, digestibility, and bioavailability as well as their biochemical composition, resistance, and stress responses [30]. Owing to soybean high protein content, plant by-products make a great replacement for fish meal are more cost-effective than cottonseed [31]. Canola meal and moringa were also frequently recommended as potential optional fish feed [32]. It has been suggested to use PM as an alternative protein source for fishmeal because it is more easily available, less expensive, and environmentally acceptable than FM due to the lower pollution-causing components of FM [33]. Many experts believe that plant by products is the ideal alternative to fish meal in fish diets since they are easily accessible year-round and are relatively less expensive [34]. Plant by-products are a great substitute for fish meal and are more cost-effective than fish meal [35].

Sesame is a plant in the genus *Sesamum*. Popular oilseed black sesame seeds are rich source of protein, amino acids, minerals, and vitamins. Sesame seeds can be used as Protein source for fish. Diets containing di-calcium phosphate (DCP) are frequently utilized to promote the health and performance of animals [36]. As *Sesame indicum* L., black sesame seeds, it is an annual general herb belonging to the Asteraceae family that has been utilized for a long time in Malaysia, India, China, and other countries as an edible oil crop [37].



Figure 1.1 Black Sesame seed

The two main minerals found in fish bones are phosphorus and calcium, which should be present in the appropriate amounts to support the fish's optimal growth [38]. Since the plant-based protein sources used to make fish feed typically contain low levels of total phosphorus, it is necessary to take phosphorus supplements to prevent shortages [39]. Fish feed supplies both phosphorus and calcium, although the amounts and bioavailability of these nutrients varied within different feedstuffs. When comparing modern aquafeeds to traditional diets, more plant sources are used [40]. Di Calcium Phosphate is a crystalline dietary supplement that adds essential minerals (phosphorus and calcium) to animals (fish) meals [41].

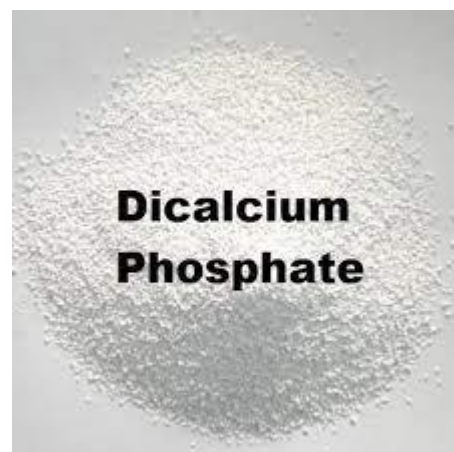


Figure 1.2 Di Calcium Phosphate

One of the main carp species farmed in Pakistan, *C. mrigala*, also referred to as "mori," is a bottom feeder that consumes decomposing organic matter and vegetable waste. To satisfy the dietary requirements of humans for high-quality fish protein, the aquaculture sector is growing very quickly [42]. One of the most well-liked Indian carps that is consumed throughout Pakistan is *C. mrigala* because of its excellent flesh quality and flavor. It is substantially distributed across Pakistan freshwater reservoirs and has substantial economic and commercial worth [43].



Figure 1.3 *C. mrigala*

AIMS AND OBJECTIVE

- Growth performance, Carcass composition and Immunological indices of *Cirrhinus mrigala* (mori) fingerlings may grow and be healthy when replacement of FM with DCP of supplemented in PM-based diets.
- To determine the greatest level of DCP supplementation in PM-based diets (as an alternative source of protein).
- To evaluate the ideal development, carcass combination, and immunological indicators of *C. mrigala* fingerlings given a PM-based diet supplemented with DCP.

CHAPTER 2

LITERATURE REVIEW

"Dietary Supplement safety" refers to people's ability to obtain enough eatable, nourishing food to lead happy and healthy lives. Increased food security is necessary for global programs to combat poverty and hunger while also promoting economic growth. The demand for food resources, especially animal protein, is rising globally due to population growth and higher consumption. Fish has long been considered a "rich food for poor people" and has been crucial in improving nutrition and food security in underdeveloped nations. Furthermore, fish can deliver up to 180 calories a day per person [44].

For 2.9 billion people worldwide, fish accounts for 15–16% of all animal protein, particularly in low-income and food-insecure countries. As more people become aware of the health benefits of eating fish, there will likely be a global demand for aquatic foods [45]. Most people believe that aquaculture is the only way to fulfill the increasing demand for aquatic foods. Around 50% of all fish consumed in 2006 came from aquaculture, up from 6% of fish produced worldwide in the 1970s. Given that fish meal has a sufficient amino acid composition and a high protein content, it has long been the main feed material used in the manufacturing of aquaculture diets [46]. Aquatic foods are affordable, practical sources of essential micronutrients and animal proteins. They are crucial to ensuring the security of food and nourishment, particularly for those who reside near the coast. These meals are becoming more and more significant because of their acknowledged substantial impact on cognitive and physical development [47].

One of the food production industries that is growing the fastest is aquaculture, it presents a potential means of meeting the growing global demand for fish. The use of fish oil and FM in carnivorous fish feed has been criticized for exploiting unsustainable fish supplies and preventing their direct use as human food, especially in less developed areas. With aquafeed accounting for over half of agricultural expenses, growers are under pressure to find more economical and sustainable solutions as fish oil and FM prices climb. The advancement of feed technology presents a viable avenue towards sustainability by enabling the production of plant-based substitutes for fish meal and oil [48].

Examined that common carp (*Cyprinus carpio* Linnaeus, 1758) During the 70day feeding trial, The diet of the fingerlings was sunflower meal supplemented with 0, 1, 2, 3, 4, and 5 mg Kg⁻¹ of selenium nanoparticles (Se NPs). Studies were done to find out how it affected growth, nutritional digestibility, and hematological parameters. Fifteen fingerlings are fed experimentally at a rate of 4% of their live wet weight in each of the three replicate tanks. Additionally, 1% of the indigestible marker chromic oxide was present in the fish. Using established procedures and methods, growth performance was assessed in terms of weight gain percentage, specific growth rate (SGR), feed conversion ratio (FCR), and nutrient digestibility (crude proteins, crude fat, and gross energy). Fish given test meal III supplemented with 2 mg Kg⁻¹ Se NPs exhibited the highest FCR (1.26), maximum weight growth percentage (210%) and nutritional digestibility (71% crude protein, 72% crude fat, and 70% gross energy). These results indicated that selenium nanoparticles at a concentration of 2 mg Kg⁻¹ should be provided to *C. carpio* fingerlings in order to enhance growth [49].

During a 70-day feeding research, the effects of canola meal-based diets enhanced with chemicals known as polyphenols on the antioxidant activity, food digestion, and growth efficiency of common carp (*Cyprinus carpio* Linnaeus, 1758) fingerlings were assessed. In total, seven trial diets were created (T0, T1, T2, T3, T4, T5, T6, and T7). The amounts of compounds called polyphenols in each diet ranged from 0 to 600 mg Kg⁻¹. Each replicate tank received thirteen fingerlings when the fish were fed each treatment meal in triplicate. Researchers investigated the treatment's impact on weight gain, nutritional digestibility, antioxidant activity, certain feed conversion ratio (FCR) and growth rate. Fish fed test meal T4 showed the highest FCR (1.31), greatest growth efficiency, and largest SGR (1.41) when fed 400 mg Kg⁻¹ of polyphenols. When 400 mg Kg⁻¹ of polyphenols were added, Relative to the crude protein (72%), the crude fat content (76%), and gross energy (67%), the proportion of nutrients that were absorbed improved significantly (p<0.05). Fish having the highest degree of antioxidant activity were those fed a T6 feed supplemented with 600 milligrams Kg⁻¹ of polyphenols. With a rise in dietary polyphenol levels, antioxidant activity rose [50].

The effects of α -tocopherol (vitamin E) on the nutritional digestibility, antioxidant activity, and growth performance of *Labeo rohita* (Hamilton 1822) fingerlings given a diet based on corn gluten meal were evaluated over a 70-day feeding study. After a seven-stage grade of α -tocopherol (0, 100, 200,300, 400, 500, and 600 milligrams Kg⁻¹

¹), the fingerlings (the average beginning weight: 6.35 g) were maintained. Each of the three similar tanks that were used could hold fifteen fingerlings. Five percent of the fish live, wet weight was fed to them. We performed a one-way analysis of variance (ANOVA) using the data that was gathered. The results showed that the fingerlings that got 200 milligrams Kg⁻¹ of α -tocopherol grew 261% of the weight of their bodies, or 32.73 g, much faster ($p < 0.05$) than the other groups. Additionally, they displayed an adequate food conversion ratio of 2.49. The fish with the highest apparent digestion coefficient (ADC%) were those fed a 200 mg Kg⁻¹ diet for gross energy (GE) (63%), ether extract (EE) (74%), and crude protein (CP) (73%), in comparison to other experimental and control diets. The antioxidant activity improved dose-dependently, as shown as the lowest level of oxidation (%) (7.66%) seen at 600 mg Kg⁻¹ [51].

The purpose of this study was to evaluate the impact of giving citric acid to fingerlings on *C. mrigala* seed meal (MOSM) with regards to their mineral absorption, hematological indices, and carcass composition. Six different experimental diets were developed by combining the baseline diet with 0, 1%, 2%, 3%, 4%, and 5% CA content. Ten fingerlings were maintained in duplicate in tanks for each treatment. For ninety days, food at five percent of the live, wet body weight was provided to their fingerlings. The inclusion of CA to the diet resulted in a significant ($p < 0.05$) improvement in the hematological indicators, carcass composition, and mineral absorption of the *C. mrigala* fingerlings as compared to a control diet. The results showed that mineral absorption was higher ($p < 0.05$) at medium levels of CA supplementation (2%, 3%, or 4% levels) than at high levels (1% and 5%). The meals supplemented with 2% and 3% of CA had the highest amounts of body crude protein and crude fats. Additionally, compared to the control diet, the hematological parameters of the fingerlings fed CA acidified feeds significantly improved ($p < 0.05$). The highest levels of MCV (187.11 fL), RBCs ($2.83 \times 10^6 \text{ mm}^{-3}$), WBCs ($7.76 \times 10^3 \text{ mm}^{-3}$), PLT (65.96), Hb (8.47 g/100 mL), PCV (24.51%), and WBCs ($7.83 \times 10^6 \text{ mm}^{-3}$) were seen in fingerlings fed a meal supplemented with 3% CA. In conclusion, *C. mrigala* fingerlings fed a meal with 3% CA acidic MOSM showed improvements in hematological indicators, carcass composition, and mineral absorption [52].

Stated that a 70-day feeding study enhanced with lycopene based on canola meal was used to examine the growth performance, nutritional digestion, and antioxidants condition of the fingerlings of *Catla catla*. Using a completely randomized design

(CRD), seven experimental meals (T1, T2, T3, T4, T5, T6, and T7) were prepared with graded lycopene levels of 0 (control), 10, 20, 30, 40, 50, and 60 mg/Kg. Fish were given food at 5 percent of their whole biomass during the trial. According to a One-Way investigation of variance, the fish that were fed a diet that contained 40 mg/Kg of lycopene had the highest weight growth percentage (193%) and the best FCR (1.95). The optimal large power (68%), protein content in crude form (75%), and ether extract (77%), of fish were all substantially distinct ($p \leq 0.05$) from diet in the control group. The test meal containing 40 mg/Kg showed the lowest rate of oxidation (3.57%), whereas the highest degree of antioxidant activity was observed in the fish given the T-V diet. It was shown that supplementing a diet mostly composed of canola meal with an adequate dosage of 40 mg/Kg of lycopene increased the development indices, antioxidant status, and nutrient utilization of *C. catla* fingerlings [53].

The present experimental study purpose was to determine how xylanase supplementation affected the *C. mrigala* mori species' capacity for growth, nutritional digestibility, and gastrointestinal enzyme activity. At baseline, Mori weighed 8.34 ± 0.23 g. Seven different trial meals were provided to them: five diets with escalating xylanase levels (680, 1273, 1882, 2486, and 3091 U/Kg), a plant-based diet (negative control, NC), and a diet heavy in fish meal (positive control, PC). Three identical groups of fifteen fish each were fed each diet for sixty days. After trial completion, the xylanase therapy significantly raised Specific growth rate (SGR), weight gain (WG), and final weight (FW) in relation to the NC diet. The ideal feed conversion ratio (FCR) for the PC diet (high fish meal content) was found to be 1882 U/Kg of xylanase supplement; this finding was not statistically significant ($P > 0.05$). Xylanase supplementation significantly affected the whole-body proximate composition for dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA), and gross energy (GE) as well as the apparent digestibility coefficient (ADC%) [54].

Elsayed *et al.* (2020) examined if PM could be used to partially replace soybean meal (SBM) in the diet of juvenile *Nile tilapia*. Five diets with the same amount of protein and energy were developed, but various quantities of PM (0%, 25%, 75%, and 100%) were included as partial replacements for SBM. A total of 180 fish were statistically placed in 30 tanks for the growth performance testing, and they were fed the appropriate diets for 90 days. The growth variables that have been assessed are as follows: weight gain percentage, length, SGR, the FCR ratio, and visible ADC%. The

study findings showed that there was no apparent difference in the fish weight gain when up to 25% of the protein that digests from SBM was replaced with PM. However, the fish carcass protein content dramatically dropped when PM was used in place of 50%, 75%, and 100% of the SBM [55].

The goal of the current study that supply fingerlings of *C. mrigala* with an economical and sustainable feed by employing Moringa oleifera leaf meal (MOLM), in addition to investigating how phytase supplementation affects nutritional digestibility and growth. MOLM was the foundation diet. In all, six test diets including phytase supplements at 0, 200, 400, 600, 800, and 1000 FTUKg⁻¹ were created. For each treatment, 15 fingerlings were divided up into three tanks. Every day, the fingerlings received feed at a rate of five percent of their wet weight. The test diet-V (800 FTU Kg⁻¹ phytase) provided the maximum weight gain (WG) of 17.95±0.1g, the lowest feed conversion ratio (FCR) of 1.13±0.01, and the highest weight gain percentage (WG%) of 248.78±2.84, according to the data. In terms of nutritional digestibility, test diet-V yielded the best results; ether extract (EE) (81.19±0.85%), crude protein (CP) (78.06±0.22%), and gross energy (GE) (67.68±0.26%) were highest in that group. Thus, this study showed that 800 FTU Kg⁻¹ was the optimal dosage for phytase supplementation, and that *C. mrigala* grew more rapidly and had higher nutritional digestibility when fed with phytase-supplemented MOLM [56].

C. mrigala, commonly referred to as "mori" is one of the main carp species raised in Pakistan. It consumes vegetable waste and decomposing organic matter since it is a bottom feeder. In order to meet the need for high-quality fish protein for human nutritional needs, the aquaculture sector is growing quickly [57]. One of the most well-liked Indian carps that is often consumed in Pakistan is *Cirrhinus mrigala* because of its excellent flesh quality and flavor. It is extensively dispersed throughout Pakistan freshwater reservoirs and has substantial economic and commercial worth [58]. *C. mrigala* accounts for over 67% of South India's total freshwater fish output, making it one of the most significant species with the highest market demand along with *Labeo rohita* and *Catla catla*. [59].

Table 2.1 Summary of efficacy of plant-based diet with supplementation of DCP studied by different researcher on different species of fish

Fish	Supplementation	Level	Optimum	Results	References
<i>Labeo rohita</i>	DCP	5,10,15,20 g Kg	5g Kg	Improve growth performance, minral absorption, nutreints digestibility.	Javid <i>et al.</i> , (2021)
<i>Siluriformes</i>	DCP	0.2_1.3%	1.3%	Beneficial effect on growth protein and lipid retention.	Kiratnikom <i>et al.</i> , (2020)
<i>Oncorhynchus mykiss</i>	DCP	5g Kg	5g Kg	Increased post prandial plasma level, whole body level.	Prabhu <i>et al.</i> , (2014)
<i>Ictalurus Punctatus</i>	DCP	0.75%	0,75%	Improved fish growth and minralization	Rabinson <i>et al.</i> , (2002)
<i>Siluriformes</i>	DCP	0,5,10,15Kg	10,15Kg	Overall growth performance.	Hung <i>et al.</i> , (2015)
<i>Nile tilapia</i>	DCP	3%	3%	Improve carcass quality	Sarkar <i>et al.</i> , (2004)
<i>Oncorhynchus mykiss</i>	phosphorus	0.5,0.4,0.8,1.2,1.6 Kg	1.6 Kg	Improved growth performance and skeletal development	Fontange <i>et al.</i> , (2009)
<i>Cyprinus Carpio</i>	Di- calcium phosphate	1%	1%	Improved growth rate of common carp	Hepher <i>et al.</i> , (1984)
<i>E. fuscoguttatus juvenile fish</i>	phosphorus	0.3,0.58,0.67,0.76,0.85,0.94%	0.67,0.76%	Enhanced growth rate	Huong <i>et al.</i> , (2024)
<i>Dicentrarchus punctatus</i>	phosphorus	0.48,0.89,0.69,1.10,1.28,1.51,1.77%	0.89,1.10%	Permoted growth performance	Zhang <i>et al.</i> , (2023)

CHAPTER 3

METHODOLOGY

3.1; STUDY SITE

To promote the health of *C. mrigala* fingerlings, an experiment will be done to substitute fishmeal with a Black sesame seed. The Fish Nutrition Laboratory, Division of Science & Technology, Department of Zoology, University of Education, Township Campus, Lahore, Pakistan is the site of the experiment.

3.2; EXPERIMENTAL DESIGN AND CONDITION

Manawa Fish Hatchery in Lahore provided 120 fingerlings that were purchased with comparable lengths and average weights. The fingerlings were kept in an aquarium with a 70-liter water capacity for the trial (as shown in Figure 3.1). The purpose of the tank was to keep experimental fingerlings. The experimental diet was formulated using a PM diet supplemented with DCP as the test ingredient. For the experiment, one hundred and twenty Mori fingerlings, with an average weight of two grams, were used. Three groups of twenty fish each will be formed at random from the fish. For eight weeks, the fish were fed. For evaluation of growth performance, carcass composition and immunological indices in *C. mrigala* fingerlings.



Figure 3.1 Fish Aquarium

3.3; EXPERIMENTAL COMPOSITION

The ingredients for each of the five diets were blended until they reach a consistent consistency. Until the time comes to feed the fish, the diets were kept at room temperature in airtight containers. six experimental tanks were set up. 70 liters of water was added to each tank. The water temperature was remained constant at 25 degrees Celsius. From a fish hatchery, 120 mori Fingerlings weighed with an average of two grams were purchased. For two days, the fish had become used to the test tank. The fish were fed for eight weeks. The fish weight was noted at the beginning and conclusion of the experiment. The weight gain was divided by the duration of the time period to determine the fish particular growth rate. The feed conversion ratio was calculated by dividing the fish feed intake by its weight growth. The digestibility coefficients for protein, crude fat, and crude fiber were obtained by feeding the fish a known quantity of each nutrient and then analyzing the amount excreted in the feces. A sample of the fish muscle tissue were analyzed to ascertain its body composition.



Figure 3.2 Oxygen Pump

3.4; EXPERIMENTAL DIET PREPARATION

The PM used in the test obtained from the Akbari Market in Lahore. As a test component, it included into the experimental diet. The PM inclusion levels of DCP in the treatment diet were 0, 0.20, 0.40, 0.60, 0.80, and 1 g/Kg. Six groups of fish were fed five experiment PM-based meals supplemented with DCP and one control meal. For about 70 days, the fish was fed the PM-based diet twice a day. In *C. marigala* fingerlings, growth efficiency measures, immunological indices, and carcass composition were measured in order to use a completely randomized design (CRD) in evaluating the PM-based diet to the control diet.



Figure 3.3 Fish Feed

3.5; FORMULATION OF FEED PELLETS

Each feed ingredient was purchased directly from the Lahore local market. The ingredients were well mixed for five to ten minutes in a blender. Fish oil and distal water were added to the mixture progressively throughout the mixing phase. The mixture that was left over was run through a mincing machine to create pellets with a diameter of 2 mm. Subsequently, the pellets were dried at 40°C in a drying chamber until their moisture content was around 10%. After being dried, the pellets were crushed into the required sizes and kept in storage at 4°C.

3.6; FEEDING PROTOCOL

The *C. marigala* fingerlings were fed a preset diet twice a day at a rate of 4% of their live wet body weight.

Table 3.1 Ingredients composition (%) of test diet

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	9.80	9.60	9.40	9.20	9
Fish oil	6	6	6	6	6	6
Vitamin/Mineral Premix*	2	2	2	2	2	2
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
total	100	100	100	100	100	100

****Vitamin (Vit.) premix per Kg:**

Vit. A 15,000,000 IU Vit. C: 15,000 mg Vit. E:30000 IU

Vit. B₂: 7000 mg Vit. B₆: 4000 mg Vit. B₁₂: 40 mg

Vit. D₃. 3,000,000 TU Vit. K₃: 8000 mg Ca pantothenate: 12,000 mg

Nicotinic acid: 60,000 mg Folic acid: 1500 mg

***Mineral premix per Kg.**

Ca: 155 g Na: 45 g P: 135 g

Mg: 55 g Cu: 600 mg Co: 40 mg

Fe: 1000 mg Mn: 2000 mg Zn:3000 mg

I:40 mg Se: 3 mg

3.7; GROWTH PERFORMANCE

The average weight of 20 fingerlings were kept in each tank. During the trial period, fish were weighed every two weeks to assess the *C. marigala* fingerlings' growth performance. Growth parameters like specific growth rate (SGR), feed conversion ratio (FCR), weight gain % of *C. marigala* fingerlings were calculated by using formula (NRC, 1993).

$$1) \quad \text{Weight gain \%} = \frac{(\text{final weight} - \text{initial weight})}{\text{initial weight}} \times 100$$

$$2) \quad \text{FCR} = \frac{\text{total dry feed intake(g)}}{\text{wet weight gain(g)}}$$

$$3) \quad \text{SGR \%} = \frac{(\text{In. final wt. of fish} - \text{In. initial wt. of fish})}{\text{trial day}} \times 100$$

3.8; IMMUNOLOGICAL INDICES

Following the trial conclusion, heparinized syringes were used to extract blood from the caudal veins of fingerlings from each diet group. Blood samples for immunological indices were brought to a laboratory. Capillary tubes and the micro-hematocrit method were used to calculate hematocrit (Brown, 1980). WBC counts were determined using a hemocytometer equipped with a recognized Neubauer counting chamber (Blaxhall & Daisley, 1973). Blood test results were collected for immunoassay inspection without the use of anticoagulants. The cells called lymphocytes the Neubauer differential counting method was used to analyze the counts of neutrophils, monocytes, and eosinophils. Serum samples were separated using the centrifugation technique, then chilled at -20°C until chemical analysis. WBC samples were determined using a **hemocytometer**

3.9; CARCASS COMPOSITION

Three fish were chosen at random from each replication, and their carcass composition was examined by killing them using accepted techniques (AOAC, 1995). The total energy in the body was estimated using an oxygen bomb calorimeter. The crude protein was examined using the Micro Kjeldahl instrument. The sample was oven dried at 105 °C for 12 hours in order to ascertain the moisture content of the carcass. The Soxhlet system was utilized to extract crude fat utilizing the petroleum ether

extraction method, while all digestion using 1.25% H₂SO₄ and 1.25% NaOH as a loss on ignition of dried lipid-free residues was used to identify crude fiber. Ash was determined at 650°C for 12 hours at constant heat in the electric furnace (Eyela-TMF 3100) Total carbohydrates was calculated using the following Formula:

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

3.10; STATISTICAL ANALYSIS

At last, an evaluation of the data concerning fish growth, hematological indices, and carcass composition was conducted using a one-way analysis of variance (ANOVA). With $p < 0.05$ significant, the mean changes were assessed using the Duckan test from the Statistical Package for Social Science (SPSS). SPSS was used for statistical analysis

CHAPTER 4

RESULTS

4.1; STUDY OF GROWTH PARAMETERS

It purchased twenty fingerlings of *C. mrigala* from Manawa Fish Hatchery in Lahore, Pakistan, with identical lengths and average weight. Supplementation of DCP with plant meal-based diets, which are less expensive, should be used in place of fish meal in fish feed. There were 20 mori fingerlings in each aquarium.

Initial weight of fingerlings in each aquarium was significantly similar with each other. From these results it was found that the initial weight of all the fingerlings was significantly similar with each other. On the other hand, fish fed test diet no. 3 (0.60g/Kg) level of DCP had the highest final weight (27 grams), weight gain (20 grams), and weight gain percentage (%) (286 grams). The fish fed test diet no. 2 (0.40g/Kg) had the second-highest values of FW (25 grams), WG (18 grams), and WG% (258%). The fish fed the control diet (0g/Kg) had the lowest final weight (21 grams), weight gain (14 grams), and weight gain % (201%). Fish fed test diet no. 5 (1 g/Kg) level of DCP had the second lowest final weight (21 grams), weight gain (14 grams), and weight gain percentage (204%). These values differed significantly from those of the other test diet. According to other test diets, the final weight was significantly different. Feed intake of fingerlings in each aquarium was significantly similar with each other. Whereas it found that highest and lowest values of FCR. whereas it found that the first highest value in the fish that were fed on control diet no (1.78) and the second highest value were fed on diet no 5 (1g/Kg) (1.65gram). Whereas found that the first lowest value in the fish that were fed on test no 3 (0.60g/Kg) (1.25) and second lowest value found in the fish that were fed on diet 2 (0.40g/Kg) (1.39gram). Whereas it was found that the highest and lowest values of SGR. Whereas found that the first highest value in the fish that were fed on test no 3 (0.60 g/Kg) (1.50) and second highest value found in the fish that were fed on diet 2 (0.40 g/Kg) (1.42). where found that the first lowest value in the fish that were fed on test diet no 0 (1.22) and the second lowest value were fed on diet no 5 (1 g/Kg) (1.23).

Table 4.1 Growth rate of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test Diets	Level of DCP g/Kg	Initial weight	Final weight(g)	Weight gain(g)	Weight gain%	W gain/70	Feed intake	FCR	survival rate	SGR
0(Control)	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
Diet-1	0.20	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
Diet-2	0.40	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
Diet-3	0.60	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
Diet-4	0.80	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±0.04 ^b
Diet-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		0.07772	0.56162	0.56009	8.41591	0.00800	0.00492	0.04625	0.86590	0.02746
Combined		1.000	0.000	0.000	.001	0.000	0.346	0.000	0.076	0.001
L		0.962	0.168	0.167	.373	0.167	0.084	0.002	0.305	0.384
Q		0.990	0.000	0.000	.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

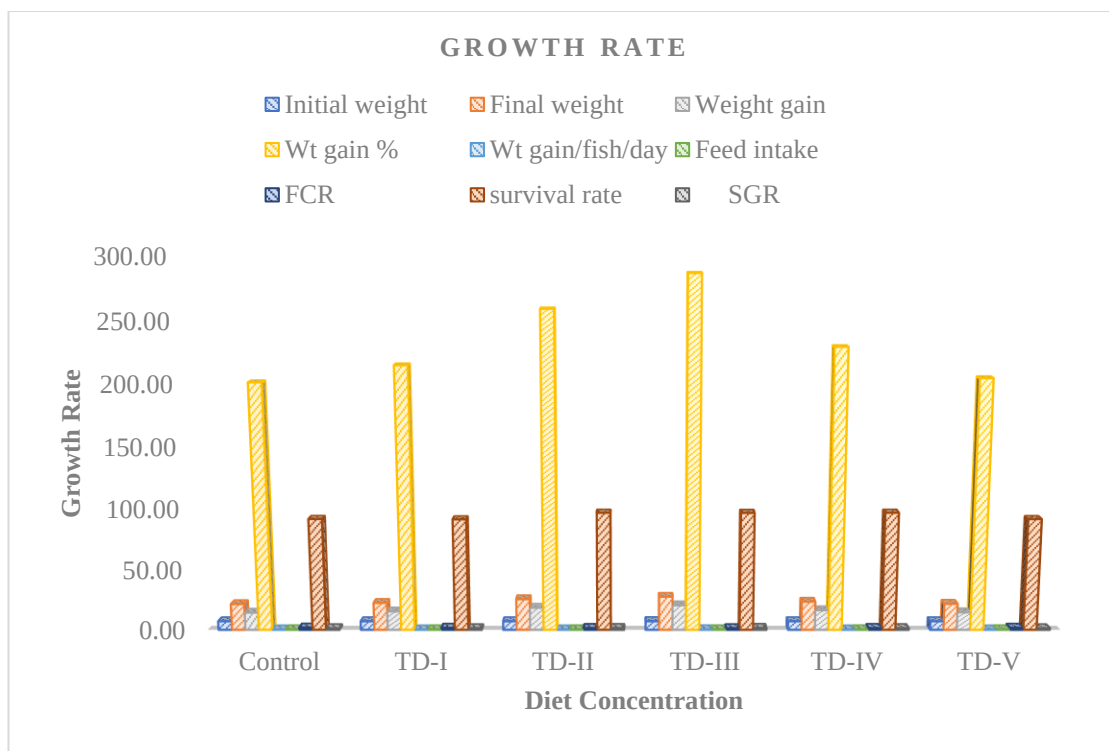


Figure 4.1 Show growth performance of *C. mrigala* fingerlings fed a diet based on PM and supplemented with DCP.

Table 4.2 Analysis of variation (ANOVA) on *C. mrigala* initial weight on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Initial weight Between Groups (Combined)	5	0.001	0.004	1.000
Contrast	1	0.000	0.002	.962
Contrast	1	0.000	0.000	.990
Within Groups	12	.779		
Total	17			

Table 4.3 Analysis of variance (ANOVA) on Final weight of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Final weight Between Groups (Combined)	5	17.435	22.386	.000
Contrast	1	1.679	2.156	.168
Contrast	1	65.138	83.638	.000
Within Groups	12	.779		
Total	17			

Table 4.4 Analysis of variation (ANOVA) on *C. mrigala* weight gain on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Weight gain Between Groups (Combined)	5	17.390	23.080	.000
Contrast	1	1.630	2.163	.167
Contrast	1	65.057	86.344	.000
Within Groups	12	.753		
Total	17			

Table 4.5 Analysis of variance (ANOVA) on Init w*100 of *C. mrigala* on the DCP supplemented plant meal-based diet.

SS	d. f	MS	F ratio	Sig.
Init wt*100 Between Groups (Combined)	5	3418.707	8.958	.001
Contrast	1	326.449	.855	.373
Contrast	1	12702.639	33.284	.000
Within Groups	12	381.642		
Total	17			

Table 4.6 Analysis of variance (ANOVA) on W gain/70 of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
W gain/70 Between Groups (Combined)	5	.004	23.080	.000
Contrast	1	.000	2.163	.167
Contrast	1	.013	86.344	.000
Within Groups	12	.000		
Total	17			

Table 4.7 Analysis of variance (ANOVA) on Feed intake of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Feed intake Between Groups (Combined)	5	.001	1.251	.346
Contrast	1	.001	3.549	.084
Contrast	1	.000	.606	.451
Within Groups	12	.000		
Total	17			

Table 4.8 Analysis of variance (ANOVA) on FCR=Feed intake/W gain of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d.f	MS	F ratio	Sig.
FCR=Feed intake/W gain Between Groups (Combined)	5	.117	20.246	.000
Contrast	1	.085	14.758	.002
Contrast	1	.439	75.904	.000
Within Groups	12	.006		
Total	17			

Table 4.9 Analysis of variance (ANOVA) on survival rate of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
survival rate Between Groups (Combined)	5	24.166	2.670	.076
Contrast	1	10.396	1.149	.305
Contrast	1	74.118	8.189	.014
Within Groups	12	9.050		
Total	17			

Table 4.10 Analysis of variance (ANOVA) on SGR of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	M S	F ratio	Sig.
SGR Between Groups (Combined)	5	.036	8.780	.001
Contrast	1	.003	.817	.384
Contrast	1	.140	33.877	.000
Within Groups	12	.004		
Total	17			

4.2; STUDY OF IMMUNOLOGICAL PARAMETERS

From Manawa Fish Hatchery in Lahore, Pakistan, 120 *C. mrigala* fingerlings with similar weights and average lengths were purchased. In fish feed, replacement of fish meal with DCP supplemented plant meal-based diet which are less costly. There were 20 mori fingerlings in each aquarium.

Immunological indices such as WBC (10^3mm^{-3}) Lymphocyte% Eosinophile% Monocyte% Neutrophile% that were studied in fish fed DCP supplemented plant meal-based diet shown in table 4.9. Results showed that the best WBC (10^3mm^{-3}) value (6.73%) was obtained when mori fingerlings were given test diet 1 (0.20 g/Kg). The second higher value of WBC (10^3mm^{-3}) (6.63%) was found in control diet 0 that par from one another. Test diet 2 had the first-lowest WBC (10^3mm^{-3}) (6.19%) value (0.40 g/Kg). Test diet 5 (1 g/Kg) had the second lowest value of WBC (10^3mm^{-3}) (6.47%). On the other hand, the highest value of Lymphocyte % (28.70%) was recorded in test diet 3(0.60 g/Kg) level of DCP. The second highest value of Lymphocyte % (27.39%) was found in test diet 2(0.40 g/Kg). The first lowest value of Lymphocyte % (19.54%) was found in test diet 5 (1 g/Kg). the second lowest value of lymphocyte % (20.33%) was found in test control diet. Whereas it found that the highest value of Eosinophile % (1.56%) in the fish that were fed on test diet no 1(0.20 g/Kg). Whereas the second highest value of Eosinophile % (1.55%) were found in the fish that were fed on test diet no 3 (0.60 g/Kg). The lowest value of Eosinophile % (1.48%) was found in the fish that were fed on control diet. The second lowest values Eosinophile % (1.49%) were found in the fish that were fed on test diet no 2 (0.40 g/Kg). Results indicated that when mori fingerlings were fed on control diet 0 had the best Monocyte % value (2.87%). The second higher value of Monocyte % (2.87%) was found in test diet 1(0.20 g/Kg). The lowest value of Monocyte % (2.09%) was found in test diet no 5 (1 g/Kg). The second lowest value of Monocyte % (2.21%) was found in test diet no 3 (0.60 g/Kg). On the other hand, fish given test diet no. 5 (1 g/Kg) had the greatest value of neutrophil percentage (76.85%). However, the fish fed the control diet had the second-highest value of neutrophil % (75.175%). Conversely, fish that were fed test diet no. 3 (0.60 g/Kg) had the lowest value of neutrophil % (67.53%). Feeding fish on test diet no. 2 (0.40 g/Kg) produced the second lowest value of Neutrophile% (68.54%).

Table 4.11 immunological indices of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test Diets	Level of DCP g/Kg	WBC (10 ³ mm ⁻³)	Lymphocyte %	Eosinophile %	Monocyte %	Neutrophile %
(Control)	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
Diet-1	0.20	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
Diet-2	0.40	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
Diet-3	0.60	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
Diet-4	0.80	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
Diet-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		0.06906	0.82895	0.03945	0.09681	0.82809
Combined		0.281	0.000	0.992	0.002	0.000
L		0.569	0.507	0.898	.001	0.106
Q		0.518	0.000	0.838	0.620	0.000

Means within columns with various superscripts (*a-d*) differ considerably at $p < 0.05$. Data are three replicates' mean (shows standard Deviations). WBC (White Blood Cells). St. E- Standard Error, L= Linear, Q= Quadratic, C= Combined

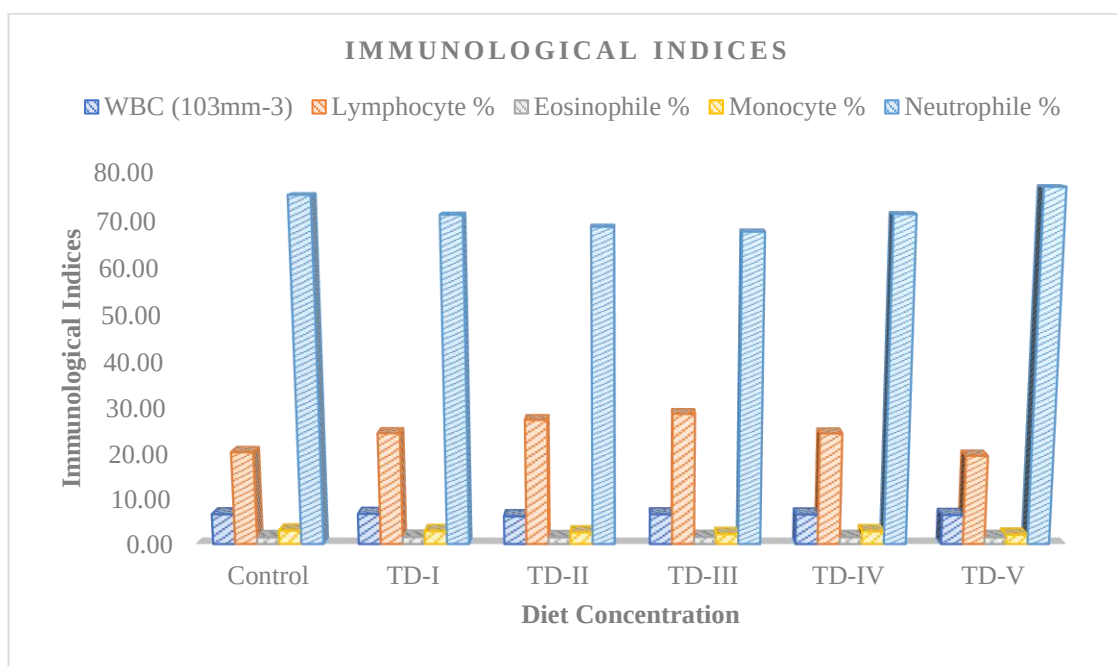


Figure 4.2 Show the immunological parameters of *C. mrigala* fingerlings fed a diet based on PM and supplemented with DCP.

Table 4.12 Analysis of variance (ANOVA) on WBC (103mm-3) immunological indices of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
WBC (103mm-3) Between Groups (Combined)	5	.109	1.435	.281
Contrast	1	.026	.343	.569
Contrast	1	.034	.445	.518
Within Groups	12	.076		
Total	17			

Table 4.13 Analysis of variance (ANOVA) on Lymphocyte % immunological indices of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Lymphocyte %Between Groups (Combined)	5	40.362	57.249	.000
Contrast	1	.330	.468	.507
Contrast	1	195.342	277.071	.000
Within Groups	12	.705		
Total	17			

Table 4.14 Analysis of variance (ANOVA) on Eosinophile % immunological indices of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Eosinophile% Between Groups (Combined)	5	0.003	0.003	.992
Contrast	1	0.001	0.001	.898
Contrast	1	0.002	0.002	.838
Within Groups	12	.038		
Total	17			

Table 4.15 Analysis of variance (ANOVA) on Monocyte % immunological indices of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Monocyte% Between Groups (Combined)	5	.438	7.732	.002
Contrast	1	1.125	19.871	.001
Contrast	1	.015	.258	.620
Within Groups	12	.057		
Total	17			

Table 4.16 Analysis of variance (ANOVA) on Neutrophile % immunological indices of *C. mrigala* on the DCP supplemented plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Neutrophile% Between Groups (Combined)	5	39.929	47.026	.000
Contrast	1	2.590	3.050	.106
Contrast	1	193.113	227.435	.000
Within Groups	12	.849		
Total	17			

4.3; STUDY OF CARCASS COMPOSITION

Carcass composition such as Protein Fat Gross energy Ash Crude fiber Carbohydrates Moisture that were studied in fish fed DCP supplemented plant meal-based diet shown in table. On the other hand, it was found that fish that were fed test diet number three (0.60 g/Kg) had the highest amount of protein (18.95%). However, the fish fed test diet no. 4 (0.80 g/Kg) (17.42%) had the second-highest value The fish fed test diet no. 1 (0.20 g/Kg) had the lowest value, however (13.17%) of the fish. The fish fed test diet no. 1 (0.20 g/Kg) had the second lowest value, however (14.0%). Results indicated that when mori fingerlings were fed on test diet 3 (0.60 g/Kg) had the highest Fat value (8.94%). The second higher value of Fat (8.47%) was found in test diet 4 (0.80 g/Kg). The lowest value of Fat (6.98%) was found in test control diet. The second lowest value of Fat (7.39%) was found in test diet no 1 (0.20 g/Kg). Whereas it was found that the highest value of Gross energy in the fish that were fed on test diet no 2 (0.40 g/Kg) (2.05%). Whereas the second highest value were found in the fish that were fed on test diet no 5 (1 g/Kg) (1.93%). Whereas the lowest value was found in the fish that were fed on control diet (1.12%). Whereas the second lowest value were found in the fish that were fed on test diet no 1 (0.20 g/Kg) (1.46%). Results indicated that when mori fingerlings were fed on control diet had the highest Ash value (6.00%). The second higher value of Ash (5.85%) was found in test diet 1 (0.20 g/Kg). The lowest value of Ash (4.93%) was found in test diet no 4 (0.80 g/Kg). The second lowest value of Ash (5.05%) was found in test diet no 3 (0.60 g/Kg). Whereas it was found that the highest value of Crude fiber in the fish that were fed on control diet (1.37%). On the other hand, fish given test diet no. 5 (1 g/Kg) had the second-highest value of crude fiber (1.33%). However, fish given Test Diet no. 3 (0.60 g/Kg) had the lowest level of crude fiber (1.14%). In contrast, the fish given test diet no. 1 (0.20 g/Kg) had the second-lowest value of crude fiber (1.18%). On the other hand, the fish given test diet number 4 (0.80 g/Kg) were found to have the highest percentage of carbohydrates (2.89%). on the other hand, the fish fed test diet no. 2 (0.40 g/Kg) had the second-highest carbohydrate (2.78%). Fish fed the control diet had the lowest carbohydrate (2.15 %), although, fish given test diet no. 1 (0.20 g/Kg) had the second lowest amount of carbohydrates (2.20%). Results indicated that when mori fingerlings were fed on control diet had the highest Moisture value (69.30%). The second higher value of Moisture (67.92%) was found in test diet 1 (0.20 g/Kg). The lowest value of Moisture

(61.17%) was found in test diet no 3 (0.60 g/Kg). The second lowest value of Moisture (63.08%) was found in test diet no 4 (0.80 g/Kg).

Table 4.17 Carcass composition of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test Diets	Level of DCP g/Kg	Protein	Fat	Gross energy	Ash	Crude fiber	Carbohydrates	Moisture
0(Control)	0	13.07±0.43 ^c	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
Diet-1	0.20	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}
Diet-2	0.40	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
Diet-3	0.60	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
Diet-4	0.80	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
Diet-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		.50356	.18405	.09249	.10803	.03433	.08748	.70867
Combined		.000	.002	.000	.001	.360	.025	.000
L		.000	.006	.000	.000	.654	.004	.000
Q		.000	.001	.000	.006	.079	.075	.000

Means within columns with various superscripts (*a-d*) differ considerably at $p < 0.05$. Data are three replicates' mean (shows Standard Deviations). DCP (Di calcium Phosphate). E- Standard Error, L= Linear, Q= Quadratic, C= Combined

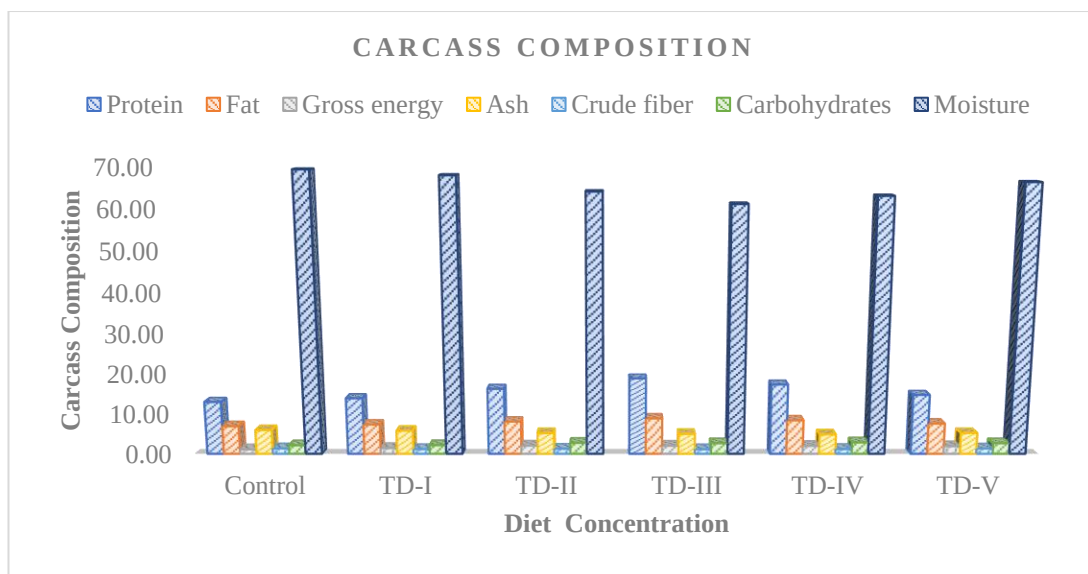


Figure 4.3 Show the carcass composition of *C. mrigala* fingerlings fed a diet based on PM and supplemented with DCP.

Table 4.18 Analysis of variation (ANOVA) on the *C. mrigala* protein carcass on a supplemented with DCP plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Protein Between Groups (Combined)	5	14.706	43.405	.000
Contrast	1	20.131	59.421	.000
Contrast	1	39.224	115.774	.000
Within Groups	12	.339		
Total	17			

Table 4.19 Analysis of variation (ANOVA) on the *C. mrigala* fat carcass on a supplemented with DCP plant meal-based diet

SS	d. f	MS	F ratio	Sig.
Fat Between Groups (Combined)	5	1.585	7.800	.002
Contrast	1	2.313	11.381	.006
Contrast	1	4.429	21.793	.001
Within Groups	12	.203		
Total	17			

Table 4.20 Analysis of variation (ANOVA) on *C. mrigala* gross energy carcass on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Gross energy Between Groups (Combined)	5	.458	16.689	.000
Contrast	1	1.396	50.886	.000
Contrast	1	.769	28.034	.000
Within Groups	12	.027		
Total	17			

Table 4.21 Analysis of variation (ANOVA) on *C. mrigala* ash carcasses on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Ash Between Groups (Combined)	5	.567	9.246	.001
Contrast	1	1.874	30.567	.000
Contrast	1	.674	10.987	.006
Within Groups	12	.061		
Total	17			

Table 4.22 Analysis of variation (ANOVA) on the *C. mrigala* crude fiber carcass on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Crude fiber Between Groups (Combined)	5	.024	1.214	.360
Contrast	1	.004	.211	.654
Contrast	1	.074	3.694	.079
Within Groups	12	12 .020		
Total	17			

Table 4.23 Analysis of variation (ANOVA) on *C. mrigala* carcass carbohydrates on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Carbohydrates Between Groups (Combined)	5	.289	3.869	.025
Contrast	1	1 .909	12.172	.004
Contrast	1	1 .284	3.801	.075
Within Groups	12	.075		
Total	17			

Table 4.24 Analysis of variation (ANOVA) on *C. mrigala* moisture carcass on a plant meal-based diet supplemented with DCP

SS	d. f	MS	F ratio	Sig.
Moisture Between Groups (Combined)	5	28.371	28.793	.000
Contrast	1	45.008	45.679	.000
Contrast	1	75.429	76.553	.000
Within Groups	12	.985		
Total	17			

CHAPTER 5

DISCUSSION

5.1; PARAMETER (GROWTH) OF FINGERLINGS (MORI) GIVEN DCP SUPPLEMENTED PLANT MEAL-BASED DIET

To determine the effect of DCP supplementation on fingerling growth, various amounts of DCP (0, 0.20, 0.40, 0.60, 0.80, and 1 g/Kg) were added to a plant-based diet. According to my research trial (FW) of the *C. mrigala* fingerlings was significantly improved growth performance by the supplemented in DCP plant-based diet. Maximum weight gain (20.40g), weight gain % (286.43%) and SGR (1.50g) values were obtained at level 3 (0.60 g/Kg) were supplementation of DCP with PM-based diet. It was also determined that mori fingerling growth was inhibited by higher DCP supplementation levels when combined with a PM-based diet. Many researchers have examined how fish development and survival are affected when DCP is supplemented with a diet based on plant meal [60]. A diet high in plant meals was thought to be a healthy source of protein [61].

Similar to current research work, Yusup & Nugroho explained the impact of DCP supplementation with a plant meal-based diet on *C. mrigala* growth performance and came to the conclusion that adding 0.75 percent of DCP to a plant meal-based diet improved *C. mrigala* development and increased weight gain, weight gain percentage, and SGR values [62]. Many 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 [63]. Similar to the studies conducted currently, fish like *L. rohita* showed lower growth performance when high inclusion levels of DCP supplementation with plant meal-based diet were used Mukhopadhyay and Ray, *tilapia* and *C. mrigala* Hasan *et al.* [64,65]. In a similar study, Nibedita substituted a plant meal-based diet for DCP supplementation in the diets of *L. rohita* fingerlings and found that greater DCP replacement levels (0 and 0.25%) led to reduced weight increase, weight gain percentage, and higher FCR [66]. Similarly, Mukhopadhyay and Ray found that fish diets using PM-based diets are unable to supplement DCP up to 0.1 and 0.27% levels, respectively, and that this leads to poor

fish growth [67]. In research that is similar to the current work, Apines-Amar *et al.* examined how DCP supplementation affected *C. mrigala* growth performance and concluded that up to 1.25% of DCP can be added to fish meals [68].

The findings of Olude *et al.* who examined the impact of supplementing DCP with a plant meal-based diet on the growth performance of *Nile tilapia*, were slightly different [69]. They determined that DCP may be added to fish diets up to 0.5% without having an adverse effect on fish growth. In contrast to current findings, some researchers observed that the FCR values of *C. mrigala* are unaffected by the 0.9% DCP supplementation with a plant meal-based diet [70].

The inconsistent findings may be caused by antinutritional components (phytic acid, tannin), low protein content, and unbalanced amino acid composition; therefore, we cannot feed fish a lot of coconut [71]. The reduced digestibility and imbalance of amino acids connected to the greater quantities of copra meal added in fish diets may be limitations and high amount of fiber [72,73].

5.2; PARAMETER IMMUNOLOGICAL INDICES OF FINGERLINGS (MORI) GIVEN DCP SUPPLEMENTED PLANT MEAL-BASED DIET

Present findings indicated that the immunological indices of *C. mrigala* fingerlings increase by supplemented in DCP plant-based diet at different inclusion levels. In current study maximum level of WBC (10^3mm^{-3}) and Eosinophile % were noticed at 1 (0.20 g/Kg) level of DCP while minimum value of WBC (10^3mm^{-3}) was obtained at 2 (0.40 g/Kg) level of DCP and Eosinophile % were noticed at control diet 0 (0 g/Kg). the amount of Lymphocyte % increase in test diet 3(0.60 g/Kg) having supplemented in DCP plant-based diets compared to control diet (without plant-based diet) in *C. mrigala* fingerlings. Whereas it was found that the highest value of Monocyte % in the fish that were fed on control diet (0 g/Kg). Whereas it was found that the highest value of Neutrophile % in the fish that were fed on test diet no 5 (1 g/Kg).

Mahboub *et al.* research, which bolsters these conclusions, showed that feeding *Nile tilapia* a diet supplemented with 0.35 g/Kg of DCP had a variety of protective benefits on the immune system [74]. Conversely, *L. rohita*, Ali *et al.* observed a significant increase in mean WBCs, particularly in those fed 0.20% DCP, highlighting the

significant impact of DCP supplementation on WBC levels [75]. Silviana *et al.* 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 1.25 g/Kg of DCP [76].

Hassan *et al.* provided support for these findings by highlighting the rise in lymphocyte levels demonstrating a spectrum of protective benefits on the immune response in *C. carpio* following a 1 g/Kg supplemented DCP-based diet [78]. A study by Hassan *et al.* found that feeding *C. carpio* a diet based on DCP caused a drop in lymphocyte numbers, which is contrary to the current findings [79]. There's still a lot to learn about fish immunology using DCP. Accordingly, Kamali *et al.* showed that adding waste date (*Phoenix dactylifera*) food to *C. carpio* resulted in a drop in lymphocyte numbers [80].

In the current investigation, at the control diet, monocyte values were raised with DCP. Conversely, Hidayat *et al.* discovered that a diet containing supplemented DCP effectively boosted non-specific immune responses, notably monocytes, at a 7.5% dosage in research including white snapper (*Lates calcalifer*) [81]. Similarly, it was discovered that adding DCP to fish diet greatly increased the quantity of monocytes [82].

5.3; PARAMETER OF CARCASS COMPOSITION OF FINGERLINGS (MORI) GIVEN DCP SUPPLEMENTED PLANT MEAL-BASED DIET

The study findings shown that adding DCP to a plant-based diet at different inclusion levels improved the composition of the carcasses of *C. mrigala* fingerlings. The current research found that the lowest values of protein, fat, and gross energy were acquired at the control diet level of DCP, while the largest levels were seen at the 0.60 g/Kg level of DCP. Research on the carcass composition of fish fed a plant-based diet supplementation of DCP has not yet been conducted. Corresponding to the present findings, Khan *et al.* noted that in Indian major carps, the amount of crude protein and fat rose in meals supplemented with DCP plant-based diets compared to control diets (without plant-based diets) [83]. According to research by Al-Souti *et al.* which found that an increase in fish oil level causes a rise in body tissue volume (14–15.1%), new study indicates that the level of protein at the first inclusion level is higher than that at

the end (high) inclusion level [84]. According to the current findings, Mukhopadhyay & Ray noted that *L. rohita* fingerlings fed with DCP supplementation at an inclusion level of 0.77% had a higher carcass composition [85].

In the current study, fish with high ash values had 0% DCP level, while fish with low ash values had 1% DCP level and 1.28% replacement of fish meal, respectively. In the present study, Fish at the 1.2% replacement of fish meal and the 0% DCP level had the lowest amounts of carbohydrates, whereas fish at the 1% DCP level had the highest levels. In contrast to recent research, Tayyab *et al.* described the impact of adding plant-based supplements to a DCP diet (sunflower, rice polish), and their results showed that as fish consume more plant foods, their ash level increased [86].

The current research work observed a high crude fiber content of 0% and a low crude fiber content of 0.60 g/Kg in the supplemented DCP plant-based diet. Given that it is commonly recognized that muscle mass rises with aging, the decline in crude fiber values that associated with an increase in DCP level suggested that the proportion of chitin and bones had decreased [87].

The current study indicated that the moisture level ranged from 0.60 g/Kg replacement to 0% replacement, which was the maximum moisture content. Other researchers agreed, speculating that the Increased protein and fat synthesis in the fish body may be the cause of the decrease in moisture content at low inclusion levels [88]. Wang *et al.* examined carcass composition in *Nibea miichthioides* by supplementing DCP in place of fish meal and found that there is an increase in moisture value at higher replacement levels [89]. This explanation was in corresponds to my findings, which indicated higher moisture values at higher inclusion levels.

A number of factors, such as different growth rates, dietary treatments containing varying amounts of nutrients (like CP, ash, and crude fiber) species differences, the variations in the carcass composition of the current research and the earlier investigations may be due to variations in fish size and the physiological capacity of farmed fish to transform meals into absorbable nutrients [90,91].

CHAPTER 6

CONCLUSION

This study aimed to identify the effects on several parameters of fingerling *C. mrigala* on a partial supplementation of DCP with a plant meal-based diet (0, 0.20, 0.40, 0.60, 0.80, 1 g/Kg). According to the current study findings, supplemented DCP at a level of 3 (0.60 g/Kg) worked best for the *C. mrigala* fingerlings growth performance, immunological indices, and carcass composition.

- Current research findings indicated that growth-related qualities, such as initial weight Final weight, weight gain, weight gain% increase W gain/70, SGR the highest results were observed in *C. mrigala* fingerlings fed on DCP supplementation at level 3 (0.60 g/Kg). These values are considerably different from the diet used as a control (which eliminates DCP). When DCP was not included in the control diet (0 g/Kg), the FCR value was at its highest. At the DCP level of diet 4 (0.80 g/Kg), the survival rate value was at its highest.
- The following immunological indices values were measured in control and test diets: WBC (103mm⁻³), Eosinophil, Lymphocytes, Monocytes, and Neutrophils. The maximum number of WBC (103mm⁻³) (6.73%) and Eosinophil (1.56%) was seen at DCP level 1 (0.20 g/Kg), but the maximum number of lymphocytes (28.70%) was discovered at DCP level 3 (0.60 g/Kg). The control diet (0 g/Kg) had the largest number of monocytes (3.02%). The maximum number of neutrophils (76.85%) was seen at the DCP level of test diet no 5 (1 g/Kg). Values of all these parameters were significantly different from those at other test.
- Carcass composition parameters related of fish such as Protein, Fat, Gross energy, Ash, Crude fiber, Carbohydrates and Moisture were improved at different diets with supplementation of DCP. The maximum number of Protein, Fat, Gross energy was discovered at DCP level 3 (0.60 g/Kg). The highest value of Crude fiber and Moisture was seen at control diet (0 g/Kg) no inclusion of DCP. Lowest values of all Protein, Fat, Gross energy and Carbohydrates parameters were found at control diet 0 (0 g/Kg) except Ash crude fiber and moisture whose lowest value was found at diet 3(0.60 g/Kg) level of DCP.

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APPENDICES

Appendix-1 Growth rate of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test diets	Level of DCP g/Kg	Replicate	Initial weight	Final weight	Weight gain	Init wt*100	Wt gain/70	Feed intake	FCR	Survival rate	SGR
0	control	R1	7.06	20.59	13.53	191.64	0.19	0.34	1.76	89.47	1.18
		R2	7.59	22.41	14.82	195.25	0.21	0.35	1.69	95	1.20
		R3	6.72	21.27	14.55	216.51	0.20	0.39	1.88	90	1.28
1	0.20	R1	7.48	22.17	14.69	196.39	0.20	0.353	1.68	88.88	1.20
		R2	7.21	23.5	16.29	225.93	0.23	0.376	1.61	94.73	1.31
		R3	6.74	21.67	14.93	221.51	0.21	0.345	1.61	90	1.29
2	0.40	R1	6.81	24.45	17.64	259.03	0.25	0.349	1.38	94.73	1.42
		R2	7.17	25.81	18.64	259.97	0.26	0.363	1.36	95	1.42
		R3	7.36	26.27	18.91	256.92	0.27	0.382	1.41	100	1.41
3	0.60	R1	7.61	27.84	20.23	265.83	0.28	0.347	1.20	100	1.44
		R2	7.18	26.57	19.39	270.05	0.27	0.359	1.29	95	1.45
		R3	6.67	28.24	21.57	323.38	0.30	0.385	1.24	94.44	1.60
4	0.80	R1	7.49	23.69	16.2	216.28	0.23	0.332	1.43	95	1.27
		R2	7.15	24.18	17.03	238.18	0.24	0.324	1.33	94.73	1.35
		R3	6.76	22.49	15.73	232.69	0.22	0.349	1.55	100	1.33
5	1	R1	7.58	20.97	13.39	176.64	0.19	0.312	1.63	90	1.13
		R2	6.81	22.41	15.6	229.07	0.22	0.348	1.56	89.47	1.32
		R3	7.03	21.61	14.58	207.39	0.20	0.363	1.74	94.73	1.24

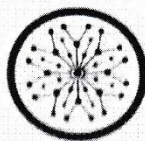
Appendix-2 Immunological indices of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test diets	Level of DCP g/Kg	Replicate	WBC (10 ³ mm ⁻³)	Lymphocyte %	Eosinophile %	Monocyte %	Neutrophile %
0	Control	R1	6.59	21.05	1.29	3.24	74.42
		R2	6.28	19.56	1.67	2.84	75.93
		R3	7.02	20.38	1.48	2.99	75.15
1	0.20	R1	7.05	23.54	1.39	3.04	72.03
		R2	6.43	25.27	1.74	2.61	70.38
		R3	6.71	24.67	1.56	2.97	70.8
2	0.40	R1	6.18	26.54	1.84	2.64	68.98
		R2	5.99	27.4	1.34	2.37	68.89
		R3	6.39	28.24	1.28	2.74	67.74
3	0.60	R1	6.74	29.67	1.64	2.18	66.51
		R2	6.32	27.88	1.47	1.99	68.66
		R3	6.8	28.56	1.55	2.47	67.42
4	0.80	R1	6.37	25.14	1.48	2.98	70.4
		R2	6.81	23.49	1.37	2.47	72.67
		R3	6.58	24.7	1.68	3.1	70.52
5	1	R1	6.39	19.65	1.72	2.01	76.62
		R2	6.27	18.7	1.34	2.31	77.65
		R3	6.76	20.28	1.51	1.94	76.27

Appendix-3 Carcass composition of *C. mrigala* fingerlings fed on supplementation of DCP with plant meal-based diet

Test Diets	Level of DCP g/Kg	Replicate	Protein	Fat	Gross energy	Ash	Crude fiber	Carbohydrates
0	Control	R1	12.69	6.61	1.01	6.01	1.36	2.3
		R2	13.54	6.99	0.96	5.81	1.48	1.74
		R3	12.99	7.34	1.39	6.19	1.27	2.41
1	0.20	R1	13.87	6.83	1.42	5.83	0.99	1.99
		R2	14.39	7.81	1.61	6.06	1.34	2.37
		R3	13.73	7.53	1.34	5.67	1.22	2.24
2	0.40	R1	15.49	8.23	1.84	4.99	1.29	3.07
		R2	16.58	7.94	2.1	5.51	1.42	2.52
		R3	17.01	8.31	2.22	5.31	1.12	2.74
3	0.60	R1	18.51	9.31	2.13	4.81	0.97	2.34
		R2	19.34	8.98	1.94	5.38	1.32	2.99
		R3	19.01	8.54	2.07	4.97	1.13	2.74
4	0.80	R1	18.34	8.61	1.84	5.11	1.31	3.09
		R2	17.14	7.98	2.19	4.71	1.2	2.64
		R3	16.79	8.81	2.02	4.96	1.08	2.93
5	1	R1	15.34	6.99	2.03	4.95	1.37	2.91
		R2	14.29	8.34	1.94	5.32	1.21	2.63
		R3	14.87	7.61	1.81	5.57	1.4	2.49

December 6, 2024



PLAGIARISM CERTIFICATE

For MPhil and PhD

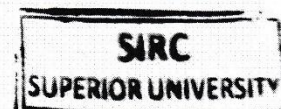
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