








Original Article

Partial replacement of fish meal with *Moringa oleifera* leaf meal in practical diets of *Cirrhinus mrigala* fingerlings

Substituição parcial da refeição de peixe com farinha de folha de *Moringa oleifera* em dietas práticas de dedos *Cirrhinus mrigala*

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Abstract

Fish protein is serving as a source of nutrition for protein starving world. However, sustainable aquaculture products require inexpensive plant by-products due to finite sources of fish meal. Therefore, this study was conducted to examine nutrient utilization, growth performance and hematological indices of *Cirrhinus mrigala* fingerlings fed on *Moringa oleifera* leaf meal (MOLM) based diets. Fish were fed with six isonitrogenous and isoenergetic diets having MOLM as a substitute of fish meal (FM) at the levels of 0%, 10%, 20%, 30%, 40% and 50% for the period of 90 days. Fingerlings having initial weight 6.35 ± 0.04 g were reared in triplicate tanks at the stocking density of 15 and hand fed at the rate of 5% of total biomass twice regularly. Chromic oxide inclusion level was 1% in diets. After analysis, maximum growth performance and improved digestibility of nutrients were found in fish fed with diet at 10% replacement level as compared to fish fed on control diet and other test diets. Additionally, it was found that the red blood cells, white blood cells, hemoglobin and mean corpuscular hemoglobin concentration of fish showed a significantly ($p < 0.05$) inverse correlation with the increase in MOLM. In present research, it was concluded that MOLM has good potential to be used as a FM substitute in *C. mrigala* diet with maximum effect at 10% showing positive hematological indices.

Keywords: *Cirrhinus mrigala*, growth performance, nutrient utilization, hematology, moringa leaf.

Resumo

A proteína do peixe está servindo como fonte de nutrição para o mundo faminto por proteína. No entanto, produtos de aquicultura sustentáveis requerem subprodutos vegetais baratos devido às fontes finitas de farinha de peixe. Portanto, este estudo foi conduzido para examinar a utilização de nutrientes, o desempenho do crescimento e os índices hematológicos de alevinos de *Cirrhinus mrigala* alimentados com dietas à base de farelo de folhas de *Moringa oleifera* (MOLM). Os peixes foram alimentados com seis dietas isonitrogênicas e isoenergéticas contendo MOLM como substituto da farinha de peixe (FM) nos níveis de 0%, 10%, 20%, 30%, 40% e 50% pelo período de 90 dias. Os alevinos com peso inicial de $6,35 \pm 0,04$ g foram criados em tanques triplicados com densidade de 15 animais e alimentados à mão a uma taxa de 5% da biomassa total duas vezes regularmente. O nível de inclusão de óxido crômico foi de 1% nas dietas. Após análise, desempenho máximo de crescimento e melhor digestibilidade de nutrientes foram encontrados em peixes alimentados com dieta em nível de reposição de 10% em comparação com peixes alimentados com dieta controle e outras dietas teste. Além disso, verificou-se que as concentrações de hemácias, leucócitos, hemoglobina e hemoglobina corpuscular média dos peixes apresentaram correlação inversa significativa ($p < 0,05$) com o aumento do MOLM. Na presente pesquisa, concluiu-se que o MOLM tem bom potencial para ser utilizado como substituto do FM na dieta de *C. mrigala* com efeito máximo a 10% apresentando índices hematológicos positivos.

Palavras-chave: *Cirrhinus mrigala*, desempenho de crescimento, utilização de nutrientes, hematologia, folha de *Moringa oleifera*.

1. Introduction

Global demand for foods in relation to aquatic origin has maximized not only because of the exponential increase in population growth, but also due to a variety

of nutritious foods for humans (Naseem et al., 2020). It is estimated that the world's population will reach 9.7 billion by the year 2050 and will heighten the supply of food by

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25-70% (Hussain et al., 2018). Approximately, 50% more food will be necessary to fulfill the increasing population demand (Diana et al., 2013). The progress of aquaculture sector is mainly dependent upon the economical feed having superior growth ability within a short period of time. The best possible way to attain low budget farming is the formulation of artificial feeds from naturally available plant stuffs (Sikotariya and Yusufzai, 2019). Conventionally, the feed formulation was based on animal protein due to its high digestibility, purity and optimal occurrence of essential amino acids, minerals and fatty acids (Olsen and Hasan, 2012). A significant quantity of FM contains a wide range of amino acids profile, which is the reason rendering it costly aqua feed ingredient. This has made it difficult for aqua-culturists to access fish meal for fish feed. Thus, the use of plant-based protein sources is being considered necessary for fish culture sector that can be totally or partially replaced fish meal in diets for fresh water fishes (Wang et al., 2016).

M. oleifera plant is a rapidly growing plant; rich in macro and micro nutrients (Oyeyinka and Oyeyinka, 2018) i.e., proteins, carbohydrates, vitamins, minerals (phosphorus, calcium, potassium, iron), beta carotene and other bioactive compounds (Sahay et al., 2017). It is easily available and can be used as a possible replacer of FM due to its high nutritional quality in fish diet (Khetran et al., 2018). The amount of crude protein in its leaves is 260g/kg of leaf, which is thought to be much higher than those of soybean seeds and other legumes (Ferreira et al., 2008). Generally, this plant is known as a multi-purpose plant and has various applications in pharmaceuticals, agriculture, livestock, human and other biological systems (Falowo et al., 2018). A study reported that its leaves contain high range of protein contents varying from 25% to 32% with low number of tannins and other anti-nutritional elements (Nouala et al., 2006).

C. mrigala, commonly known as "Mori", is one of the major carp species; widely distributed in freshwater reservoirs of Pakistan. It has considerable economic

importance and market value. This species is basically a bottom feeder, feeds on plant debris and decomposed organic matter, and can be grown with other major carp species as well as with Chinese carps (Hussain et al., 2011). It is most preferable for consumers because of its taste quality and highly nutritious value. In past, researches revealed that there is unavailability of literature as a replacer of FM by MOLM based diet for commercially important major carp species including *C. mrigala*. Hence, objectives of the present research encompass focusing on finding the best and cost effective protein source (MOLM) for *C. mrigala* fingerlings to improve its growth performance and to evaluate the nutrient utilization and hematological indices of *C. mrigala* fed varying inclusion levels of MOLM based diets.

2. Materials and Methods

The current research was performed in the Fish Nutrition Laboratory at the Department of Zoology, Government College University Faisalabad, Pakistan (Latitude 31.4166° North; Longitude 73.0707° East) for the period of 90 days.

2.1. MOLM processing and diet formulation

Fresh *M. oleifera* leaves were harvested from the Southern Punjab, Multan and underwent various processing stages. Preliminary step was to soak it in water overnight; so, to remove soluble anti-nutritional factors (Wee and Wang, 1987). Furthermore, leaves were air dried and grinded enough to get fine powder and stored in plastic zipper bags. All the feed ingredients were bought from a commercial feed mill and grinded to pass through sieve mesh (size 0.5 mm). Diet composition of nutrients showed that there was an equal amount of nutrients in all the MOLM based test diets including the control diet as shown in Table 1. First of all, grinded ingredients were analyzed for chemical composition (table 2) following standard methods (AOAC,

Table 1. Ingredients and proximate composition (%) of test diets.

Ingredients	T ₁ (Control)	T ₂	T ₃	T ₄	T ₅	T ₆
Replacement Levels	0%	10%	20%	30%	40%	50%
MOLM	0	10	20	30	40	50
FM	50	44	38	32	26	20
Wheat flour	24.50	20.50	16.50	12.50	8.50	4.50
Corn gluten (60%)	16.6	16.6	16.6	16.6	16.6	16.6
Fish oil	4.9	4.9	4.9	4.9	4.9	4.9
Vitamin Premix	1	1	1	1	1	1
Mineral Premix	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Proximate composition of test Diets						
CP%	30.84±0.02 ^a	30.86±0.03 ^a	30.85±0.03 ^a	30.86±0.02 ^a	30.85±0.02 ^a	30.84±0.02 ^a
CF%	7.04±0.02 ^a	7.03±0.02 ^a	7.04±0.02 ^a	7.03±0.01 ^a	7.03±0.02 ^a	7.04±0.02 ^a
GE (kcal g ⁻¹)	3.53±0.01 ^a	3.54±0.02 ^a	3.54±0.03 ^a	3.53±0.02 ^a	3.54±0.02 ^a	3.54±0.02 ^a

Data are means of three replicates (± shows Standard Deviations). CP= Crude protein, CF= Crude fat, GE= Gross energy.

Table 2. Chemical composition (%) of feed ingredients on dry matter basis.

Ingredients	MOLM*	FM**	Rice polish	Wheat flour	Corn gluten meal (60%)
Dry Matter (%)	93.21	92.17	95.01	93.37	91.98
Crude Protein (%)	26.47	46.36	13.34	10.54	54.12
Crude Fat (%)	5.45	6.85	12.96	2.24	3.98
Crude Fiber (%)	7.55	1.19	13.01	3.40	1.47
Ash (%)	11.35	24.04	11.21	2.77	1.87
Gross Energy (kcal/g)	3.29	4.02	2.83	2.51	4.84
Carbohydrates	45.89	17.54	46.65	78.54	33.72

*MOLM= *M. oleifera* leaf meal. **FM= Fish meal

2005). After mixing it thoroughly, fish oil was added as a source of fats. Cr₂O₃ (1%) was used as an inert marker in the test diets. During mixing of ingredients, 15% moisture level was ensured (Lovell, 1989). After drying and making pellets, feed was packed in plastic jars and stored at 4°C.

2.2. Test species and experimental conditions

C. mrigala fingerlings were acquired from Government Fish Seed Hatchery, Faisalabad, Pakistan. The fingerlings with mean weight of 6.35±0.04g were acclimatized for 14 days prior to feed the test diets. Water tanks were particularly made in V-shape, containing capacity of 70L water. During this acclimation stage, fingerlings were hand fed once regularly on the basal diet to apparent satiation (Allan and Rowland, 1992). Before the initiation of feeding experiment, *C. mrigala* fingerlings were treated with 0.5% saline solution for a couple of minutes, so to remove from the pathogens, if existed (Rowland and Ingram, 1991). Water temperature (25.0 ± 0.8 °C), pH (7.5-8.5) and dissolved oxygen (6.0 ± 0.9 mg/L) were kept adjusted throughout the feeding trial. Fish were supplied with proper aeration through capillary system.

2.3. Experimental design and feeding protocol

MOLM was used as test ingredient and included in six test diets by replacing FM at levels of 0%, 10%, 20%, 30%, 40% and 50% as shown in Table 1. A total of 270 fingerlings were divided into six groups; with stocking density of 15 fingerlings in triplicate water tanks. Diets were allocated to each group following Completely Randomized Design (CRD). Fingerlings were fed at the rate of 5% of total fish biomass. After 2 hours of feeding, fecal material was collected carefully to avoid the breakage of thin fecal strings so that leaching of nutrients in water could be lessened. Finally, feces were dried in oven at 65°C for 4 hours, ground and then stored in air-tight boxes for further chemical analysis.

2.4. Chemical analysis of feed and feces

For chemical analysis, crude protein (CP) (N × 6.25) was determined by using micro Kjeldahl apparatus whereas crude fat (CF) was extracted with the help of petroleum ether extraction method using Soxhlet system. Gross energy (GE) of samples was estimated by using adiabatic

oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). Total carbohydrates (N-free extract) were calculated by difference i.e.

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

2.5. Nutrient digestibility calculation

After oxidation with molybdate reagent, Cr₂O₃ content of the experimental samples were calculated by using acid digestion method (Divakaran et al., 2002) by the help of UV-VIS 2001 Spectrophotometer at 370nm absorbance. Apparent nutrient digestibility coefficients (ADC%) for experimental diet were calculated by the standard formula reported by NRC (1993).

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

2.6. Calculation of growth indices

Fish was weighed once at the beginning and secondly at the end of study after starvation. Growth performance of fingerlings was evaluated using standard formulae (NRC, 1993).

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

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

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

2.7. Hematological analysis

Initially, anesthesia with clove oil (Sigma) concentration of 60 mg/L was given to *C. mrigala* for 5 minutes at the end of the experiment; to collect blood samples from the caudal vein of fish. Samples of blood were taken to the Molcare Lab at the Department of Biochemistry in University of Agriculture, Faisalabad, Pakistan for analysis of hematology. The hematological indices i.e., RBCs, WBCs, Hb, MCV, MCH and MCHC were determined by using automated cell counter. PLTs were measured as

described by Brahimi et al. (2009). PCV was measured in a centrifuge at 10,500 rpm using micro-hematocrit reader. For hematological analysis, MCV, MCHC and MCH were determined by following formulae:

$$MCV = (PCV / RBC) \times 10$$

$$MCHC = (Hb / PCV) \times 100$$

$$MCH = (Hb / RBC) \times 10$$

2.8. Statistical analysis

Finally, data of ADC% of nutrients, growth parameters and hematological indices was subjected to one-way analysis of variance (Steel and Torrie, 1996). The differences among diets were compared by Tukey's Honest Significant Difference Test and considered significant at $p < 0.05$ (Snedecor and Cochran, 1991). For statistical analysis, the CoStat Computer Package (Version 6.303, PMB 320, Monterey, CA, 93940 USA) was used.

3. Results

3.1. Apparent nutrient digestibility

After 90 days of feeding trial, it was clear that minimum amount of nutrients was discharged through feces in water at 10% MOLM followed by 20% replacement level (Table 3). It was also evident from the results that fish group fed on T_2 showed maximum digestibility value for crude protein (68%), crude fat (77%) and gross energy (58%) as illustrated in figure 1. These values showed significant ($p < 0.05$) increase in nutrient digestibility for *C. mrigala* fingerlings at 10% MOLM replacement-based diet in comparison with control diet. Interestingly, it was also apparent that remaining diets except 10% and 20% replacement levels could not show increase in CP, CF and GE digestibility. Fish diet having inclusion levels of MOLM up to 30% showed CP; 60%, CF; 68% and GE; 47%, which was nonsignificantly ($p > 0.05$) different than that of control diet (62%, 71%, 50%).

3.2. Growth indices

The results obtained for growth performance showed that MOLM based test diets maximized the growth quality indices of *C. mrigala* fingerlings as compared to

the control diet. From the findings, a significant ($p < 0.05$) enhancement in WG (g), WG (%), SGR (%/day) and FCR was noted (14g, 225%, 1.31%/day and 1.46), respectively by using T_2 having 10% MOLM (Table 4). Adding 30%, 40% and 50% levels of MOLM significantly decreased weight gain (%) of *C. mrigala* fingerlings as 153%, 140% and 118%, which is much lower than that of fish fed on control diet (176%). FCR values were also maximum (2.06, 2.22, 2.54, respectively) on these levels showing less significant effects on fish growth.

3.3. Hematological parameters

The hematological indices (RBCs, WBCs, Hb and MCHC) of *C. mrigala* fingerlings fed MOLM based diet showed a decreasing trend as MOLM increased in the diet (Table 5). The increased values of RBCs ($2.50 \times 10^6 \text{ mm}^{-3}$), WBCs ($7.50 \times 10^3 \text{ mm}^{-3}$), Hb (8.47 g/100 ml) and MCHC (33.94%) were noted in fish fed on T_2 , which differed significantly ($p < 0.05$) from control group and T_3 and T_6 diets. Moreover, blood indices such as PCV increased (26.46%) with the increase in MOLM level up to 20%, afterwards it showed a declined trend. In addition to it, PLT, MCH and MCV showed that the fish fed with diet containing 50% MOLM had the highest values of 65.77, 69.71 pg and 161.62 fl, respectively, whereas the least values of PLT (50.47) were noticed in fish fed on control diet and least values of MCH and MCV (25.37 pg) and (85.53 fl) were found in fish fed on MOLM 10% diet.

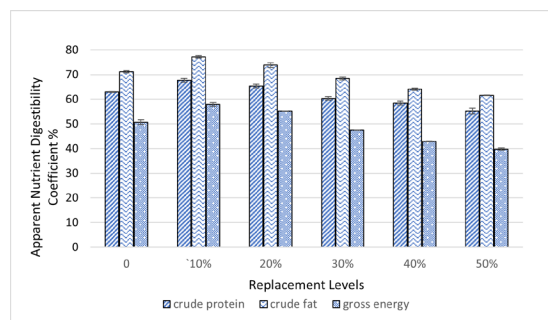


Figure 1. Apparent nutrient digestibility coefficient (%) of *C. mrigala* fingerlings fed on MOLM based test diets

Table 3. Analyzed composition of (CP, CF and GE) in the feces of *C. mrigala* fingerlings fed on MOLM based test diets

Test Diets	Replacement Levels of MOLM (%)	CP (%)	CF (%)	GE (kcal g ⁻¹)
T_1 (Control)	0	11.94±0.03 ^c	2.12±0.03 ^c	1.82±0.03 ^c
T_2	10	10.49±0.02 ^a	1.69±0.04 ^a	1.57±0.02 ^a
T_3	20	11.36±0.02 ^b	1.96±0.03 ^b	1.69±0.04 ^b
T_4	30	12.95±0.04 ^d	2.35±0.04 ^d	1.96±0.03 ^d
T_5	40	13.49±0.04 ^e	2.66±0.04 ^e	2.12±0.03 ^e
T_6	50	14.56±0.04 ^f	2.85±0.04 ^f	2.25±0.04 ^f

Means within columns having different superscripts are significantly different at $p < 0.05$. Data are means of three replicates (\pm shows Standard Deviations). CP= Crude protein, CF= crude fat, GE= Gross energy

Table 4. Growth Parameters of *C. mrigala* fingerlings fed on MOLM based test diets.

Test Diets	Replacement levels of MOLM (%)	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Weight Gain (%)	Weight Gain (fish ⁻¹ day ⁻¹)g	Feed Intake (fish ⁻¹ day ⁻¹)g	FCR	SGR (% day ⁻¹)
T ₁ (Control)	0	6.35±0.03 ^a	17.57±0.75 ^c	11.21±0.77 ^c	176.52±0.69 ^c	0.12±0.01 ^c	0.23±0.00 ^{ab}	1.83±0.12 ^{bc}	1.13±0.05 ^c
T ₂	10	6.34±0.03 ^a	20.63±0.76 ^a	14.29±0.74 ^a	225.42±0.66 ^a	0.16±0.01 ^a	0.23±0.00 ^a	1.46±0.07 ^a	1.31±0.04 ^a
T ₃	20	6.36±0.04 ^a	19.05±0.24 ^b	12.69±0.21 ^b	199.68±0.21 ^b	0.14±0.00 ^b	0.23±0.00 ^{ab}	1.62±0.04 ^b	1.22±0.01 ^b
T ₄	30	6.35±0.04 ^a	16.07±0.18 ^d	09.73±0.15 ^d	153.25±0.44 ^d	0.11±0.00 ^d	0.22±0.00 ^{bc}	2.06±0.03 ^{cd}	1.03±0.01 ^d
T ₅	40	6.34±0.04 ^a	15.24±0.43 ^d	08.90±0.47 ^d	140.34±0.36 ^d	0.10±0.01 ^d	0.22±0.00 ^c	2.22±0.13 ^{de}	0.97±0.04 ^d
T ₆	50	6.35±0.04 ^a	13.87±0.29 ^e	07.52±0.30 ^e	118.38±0.12 ^e	0.08±0.00 ^e	0.21±0.00 ^d	2.54±0.12 ^e	0.87±0.03 ^e

^{a-f} Means within columns having different superscripts are significantly different at p<0.05. Data are means of three replicates (± shows Standard Deviations). SGR=Specific Growth Rate, FCR= Feed Conversion Ratio.

Table 5. Hematological parameters of *C. mrigala* fingerlings fed different levels of MOLM based test diets.

Test Diets	Replacement Levels of MOLM (%)	RBC (10 ⁶ mm ⁻³)	WBC (10 ³ mm ⁻³)	PLT	Hb (g/100ml)	PCV (%)	MCHC (%)	MCH (pg)	MCV (fl)
T ₁ (Control)	0	2.17±0.02 ^c	7.26±0.02 ^c	50.47±0.02 ^f	7.46±0.03 ^c	26.03±0.02 ^b	29.96±0.02 ^c	26.51±0.02 ^d	95.45±0.03 ^e
T ₂	10	2.50±0.01 ^a	7.50±0.01 ^a	58.96±0.02 ^e	8.47±0.02 ^a	25.47±0.03 ^c	33.94±0.05 ^a	25.37±0.02 ^f	85.53±0.03 ^f
T ₃	20	2.24±0.03 ^b	7.33±0.02 ^b	63.85±0.02 ^c	7.95±0.03 ^b	26.46±0.03 ^a	31.77±0.02 ^b	25.96±0.03 ^e	96.54±0.03 ^d
T ₄	30	1.36±0.03 ^d	7.19±0.02 ^d	61.75±0.03 ^d	7.37±0.02 ^d	22.91±0.03 ^d	26.95±0.03 ^d	50.87±0.02 ^c	113.17±0.02 ^c
T ₅	40	1.26±0.02 ^e	7.09±0.02 ^e	64.35±0.02 ^b	7.24±0.03 ^e	20.75±0.02 ^e	24.86±0.03 ^e	62.47±0.03 ^b	129.97±0.02 ^b
T ₆	50	1.15±0.03 ^f	7.03±0.02 ^f	65.77±0.02 ^a	7.09±0.02 ^f	20.43±0.02 ^f	23.51±0.02 ^f	69.71±0.02 ^a	161.62±0.03 ^a

RBC = Red Blood Cell, WBC = White blood cell, PLT = Platelet, Hb = hemoglobin, (PCV = Packed cell volume, MCHC = Mean corpuscular hemoglobin concentration MCH = Mean corpuscular hemoglobin, MCV = Mean corpuscular volume). Means within columns having different superscripts are significantly different at p < 0.05. Data are means of three replicates.

4. Discussion

Global population is depending on fish as an essential source of protein on account of its cost (Agbo et al., 2014). So, the demand of fish has been increased due to the rapid growth in fish consumption (Hussain et al., 2018a). Moringa plant is a miracle tree; have high protein level, good taste and abundant nutrients. It has been reported that MOLM can be considered as a potential feed ingredient of fish feed to make aquaculture production cost effective due to its wide availability throughout tropical and sub-tropical regions (Tagwireyi et al., 2014). Being an innovative feed stuff, it is being used to overcome the feed crisis (Su and Chen, 2020).

The nutrient utilization and growth performance of fish was first increased up to 10% level, while later on decreased as MOLM inclusion levels increased in diets. This is due to constant increase in the replacing levels of FM with MOLM which could stop growth in fish as reported by various researchers (Richter et al., 2003; Ozovehe, 2013). They further elaborated that MOLM can be used as a source of dietary protein up to 10% in *Oreochromis niloticus* and *Clarias gariepinus*. According to Madalla et al. (2013), the possible reason of reduced nutrients of the fish body can be the starvation; which occurred due to poor feed intake at higher inclusion levels of MOLM. Ultimately, the fish is compelled to use body fat reserves for vital functions

of the body. Results of growth performance of rainbow trout were in agreement with our research, in which Labh (2020) stated improved weight gain (349%), FCR (1.33) and SGR (1.35) values by using 20% inclusion level of MOLM in fish diet. At the same level, enhanced fish immunity and antioxidant status was also noted. Agreeing results of improved growth indices were obtained by El-abd et al. (2019) by using 1.5% MOLM based diets for *O. niloticus*. It can be said that moringa affects growth parameters positively.

Similarly, Hussain et al. (2018) found optimal level of MOLM inclusion level is 10%; so, to achieve highest growth parameters in *Labeo rohita* and maximum apparent digestibility coefficients (ADC%) of nutrients. They concluded that use of 10% MOLM in place of FM is optimal range at which maximum nutrient digestibility is obvious and suitable amount of chelated nutrients were released out to utilize by fish body. Moringa leaf meal successfully changed the crude protein and crude ash of sea bass at different inclusion levels (Ganzon-Naret, 2014). Inclusion of MOLM at lower replacement level is suitable for mono-gastric animals while higher replacement levels show deleterious effects on nutrient digestibility (Kakengi et al., 2007). It could be due to the presence of anti-metabolites (tannins, phenol, saponins and phytates) contained in MOLM based diets which retards growth response in fish and ultimately pancreatic activity of lipase is inhibited. Thus, dietary nutrients are less absorbed in

gastrointestinal tract (Han et al., 2000). Bisht et al. (2020) also found non-significant effects of moringa leaf powder up to 15% on growth performance of *Poecilia reticulata*. It may be due to increased content of saponins and phytic acid contents which lead to physiological disturbance in the fish.

Hematological indices are usually considered as a main indicator for examining the health condition of fish (Hrubec et al., 2000). The past research directed by Osuigwe et al. (2005) highlighted that hematological indices in fishes vary due to numerous aspects such as age, size, physiological responses as well as environmental status. In our study, *C. mrigala* fed on MOLM based diet at 10% replacement level showed the maximum values of RBCs ($2.50 \times 10^3 \text{ mm}^{-3}$), WBCs ($7.50 \times 10^3 \text{ mm}^{-3}$), Hb (8.47 g/100 ml) and MCHC (33.94%), afterwards it decreased with the increase in MOLM concentration. Similarly, Adeshina et al. (2018) recommended 10% level of MOLM to be incorporated in the diets of common carp at juvenile stage; for maximum growth performance and hematological indices like increased amount of Hb ($4.80 \pm 0.12 \text{ g/dL}$) and RBCs ($1.07 \times 10^6 \text{ mm}^{-3}$). These indicate the improved oxygen carrying capacity of the blood. In accordance to our results, El-Gawad et al. (2020) recorded a considerable increase in WBCs ($12.07 \times 10^4/\mu\text{l}$) while feeding 1.5% moringa leaf powder. While RBCs and Hb count was not increased significantly in the Nile tilapia. This is contradiction to our work and the other researchers may be due to dose level, fish species and its size, time of allocation of diets and fish health status.

Similarly, Bello and Nzeh (2013) and Arsalan et al. (2016) also found declining trend in RBCs with the increase in MOLM levels in the diets of *C. gariepinus* and *L. rohita*, respectively. Reason could be the higher concentration of anti-nutritional elements particularly presence of tannin in the fish diets having more inclusion of MOLM in fish diet. In current research, WBCs increased up to 10% replacement level, above that it showed decreasing trend. The study of Douglass and Janes (2010) highlighted that WBCs play a key role in immune responses and enhances the proficiency of the animal to fight against infection.

5. Conclusion

It depicted that inclusion of MOLM at 10% is favorable to gain highest nutrient utilization, maximum growth performance; without disturbing health status of *C. mrigala* fingerlings. Results of the study indicated that the inclusion of MOLM at higher replacement levels had an apparent adverse effect on health status of *C. mrigala* fingerlings. Furthermore, it was inferred that costly FM can be replaced with MOLM up to 10% without any adverse effects on fish nutrient utilization, growth performance and hematological indices.

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