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Full Length Article

Effects of Mucuna Leaf Meal (*Mucuna bracteata*) with Blood Parameters, Immune Response and Antioxidant Enzyme Activities *Cyprinus carpio* (Linnaeus 1758)

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Abstract

Changes in blood parameters, immunological responses and, activation of antioxidant enzymes of *Cyprinus carpio*, the fingerlings are being fed *Mucuna bracteata* leaf meal (MLM) as a rice bran substitute (DORB) were investigated in this study. The trial, which lasted eight weeks, used a total of hundred and eighty fingerlings. In a Completely Randomized Design, four treatment groups were randomly selected, each with fifteen (15) fingerlings and each treatment was reproduced three times (CRD). The following treatments were created using four isonitrogenous (32 percent crude protein) and isocaloric (356 kcal DE/100 g) diets, control (C) had no MLM, but MLM was utilized to replace DORB at 10 percent (T1), 20% (T2) and 30% (T3), respectively. Both muscle and liver alanine aminotransferase (ALT) activities were considerably elevated ($P < 0.05$) in the MLM 20% group. There were also significant increases in hepatic superoxide dismutase (SOD) and catalase activity ($P 0.05$) in the MLM20 and MLM30 groups. There was no significant difference between treatments in serum albumin concentration, total protein, or the ratio albumin/globulin ($P > 0.05$). MLM20% and MLM30% groups had substantially higher Nitroblue Tetrazolium (NBT) values than the other MLM fed groups ($P < 0.05$). As a result, the study concludes that mucuna leaf meal derived from *M. bracteata* can fully replace DORB in the diet of *C. carpio* without causing any negative effects, while 20% MLM inclusion offered additional benefits. © 2022 Friends Science Publishers

Keywords: Mucuna Leaf meal; Super oxide dismutase; De-oiled rice bran; Catalase; Aminotransferase

Introduction

Aquaculture contributes significantly to poverty reduction and nutritional security, hence increasing impoverished people's socioeconomic condition, particularly in developing nations (FAO 2018). In 2018, global fish production reached 179 MMT, with 82 MMT produced from the aquaculture industry (FAO 2020). Increased feed-based aquaculture practices allowed for this expansion. Aquafeeds must combine unproven unconventional locally available components because a source of protein and energy typical dietary items are currently in short supply. Traditional feedstuffs for carp production in Asia include oil cakes and deoiled rice bran (DORB), which is mostly utilized in aquafeeds (Meshram *et al*. 2018). Farm-made carp feed, on the other hand, typically contains more than 85 percent DORB. As a result, fish feed made in farms is highly dependent on DORB may pose challenges about availability in the future. The use of DORB in human food and the terrestrial livestock feed industry may also result in decreased accessibility to DORB as an aquafeed ingredient (Maiti *et al*. 2019). Thus, alternatives to DORB have to be identified. The unused and wasted plant leaves in the form of dried meal may be a suitable option for DORB replacement in the fish diet (Meshram *et al*. 2018; Ahmad *et al*. 2019; Maiti *et al*. 2019; Sahoo *et al*. 2020; Anand *et al*. 2020).

Due to the existence of antinutritional influences, plant leaves have limitations for optimal inclusion in aquafeed (ANFs). Sweet potato, *Leucaena leucocephala*, *Morus esculenta*, Medicago sativa, *Moringa oleifera*, and *Manihot esculenta* are just a few of the plant leaves that have lately been added into fish diets (Ali *et al*. 2003; Bairagi *et al.* 2004; Mondal *et al.* 2012; Diarra *et al.* 2017; Meshram *et al.* 2018), with promising results. To ensure the availability around the season and geographical locations, a greater number of leaf meals should be evaluated in aquafeed. The current study was intended to evaluate the potential of introducing mucuna common carp pellets are enriched with leaf meal; there is a widespread cultured Species of fish found throughout the world. The nutrition of the Mucuna bean (*Mucuna pruriens* var. Use), grains legumes native to tropical regions, a similarity can be found between soybeans

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and other legumes commonly found in agriculture, as it includes the same levels of nutrients, such as protein, fat, minerals and vitamins (Siddhuraju *et al.* 2000). According to Duke (1981), its value as a source of nutritional protein for animal feeds is well known, especially in underdeveloped nations. *Cyprinus carpio* L., sometimes known as common carp, is an economically important freshwater fish species that accounts for 8% of global finfish aquaculture production (FAO 2018). Under a variety of geographical, meteorological and technical conditions, it is the world's third most farmed species (Safari *et al.* 2016). Since its farming becomes common in tropical areas, it's essential to evaluate cheaper feed resources like leaf meals in the feed of this commercially important species.

Materials and Methods

Experimental details and treatments

Leaf meal preparation: *M. bracteata* DC. ex (Access no: 14825) leaves were collected from Calicut, Kerala. Tap water was used to remove the dirt from the leaves contaminants and obtain the lowest amount of moisture without altering the profile of nutritional intake., Then it was dehydrated for two days in a shaded place. Dried leaves were grounded with a mixer-grinder, kept in an airtight container in ICAR-CIFE's feed lab.

Experimental diets: Four isonitrogenous and isocaloric diets (32% CP and 356 kcal DE/100 g) were prepared by including Mucuna leaf meal (MLM) at 10, 20 and 30% in the diet, intended to replace DORB at 33.3, 66.6 and 100%. DSBM, GNOC, MLM, WF, DORB and CMC were weighed accurately (Table 1) and mixed to create a homogeneous mixture. At 121°C, the dough was steam-cooked for 25 min. Oil, BHT, after the dough had cooled; vitamin-mineral premix and choline chloride were combined. An automatic pelletizer was used to pelletize the dough (SB Panchal Company). After one day at room temperature, the pellets were dried under a fan, then for one hour in a 40°C oven. After that, the pellets samples were allowed to dry for one day at room temperature under a fan, following that, it was dried for one hour using a 40°C oven, before being cooled and stored until needed at 4°C in sealed containers.

Proximate analysis of leaf meal and diets

At the Fish Nutrition Laboratory, CIFE, dietary proximate analysis was carried out utilizing conventional techniques (AOAC 1995). By removing the weight before and after an overnight drying at 105°C, the moisture content was calculated. The crude protein content % and crude lipid content % were determined using Micro-Kjeldahl and Soxhlet extraction. A muffle furnace kept at 450°C was used to ash the feed and ingredients and the crude ash content (%) was estimated after weighing the ashes residue. Acid and alkali digestion were used to calculate the crude fiber content (CF%). Nitrogen free extract was computed by the formula:

NFE % = 100 – [Crude protein (%) + Ether extract (%) + Crude fibre (%) + Total ash (%)].

Antinutritional factors (ANFs)

The Vaintraub and Lepteva (1998) spectrophotometric approach were used to measure the phytic acid content in MLM. According to Makkar *et al*. (2007) total tannin was determined using a spectrophotometric approach. Day and Underwood (1986) spectrophotometric method for estimating total oxalate was used. The gravimetric method was used to determine the amount of alkaloids in leaf meal. The saponin content of the leaf was determined using a technique developed by (Hiai *et al.* 1976).

Experimental animal procurement and acclimation

The fingerlings of *C. carpio* were sent to the ICAR-CIFE wet laboratory facility in Mumbai, where two FRP circular tanks were with constant aeration. A control diet was given to the fish for 21 days to acclimate them (31 g crude lipid 100 g^{-1} and 6 g lipid 100 g^{-1}).

Experimental design

The present study used a completely randomized design. One hundred and eighty acclimated *C. carpio*, in triplicates, fingerlings (average bodyweight 6.06 - 0.08 g) were assigned to four experimental groups at random. The feeding trial lasted 60 days and took place at the ICAR-wet CIFE's laboratory in Mumbai, India, (tank measurements: 57x 36 x 47 cm, 150 L water volume, 175 L capacity). Fish in each experimental tank were fed up to satiation using the respective experimental diet two times a day (9 AM and 5 PM). Each experimental tank's fecal matter was siphoned out and replaced with an equal volume of siphoned water before the feeding for the next day began in the morning.

Estimation of metabolic and oxidative stress enzymes

Following the completion of the experiment, each replicate had two fish anesthetized with 50 l/L clove oil and the liver and muscle were dissected and a mechanical homogenizer was used to prepare using a cold sucrose solution (0.25 *M*), homogenize the tissue. The whole thing was done in an ice bath, and the homogenates were centrifuged for 10 min at 5000 rpm. The supernatants had been accumulated and stored in vials at 20°C.

The activity of alanine aminotransferase (ALT) was tested similarly AST, for example, with exception of the substrate. AST in muscle and liver was measured and as defined by Wooten (1964), nanomoles of produced oxaloacetate/mg protein/min (1964). Including the substrate, the action of alanine aminotransferase (ALT) was measured

correspondingly to the same AST. Nanomoles of pyruvate generated per milligram of protein every minute was used to express ALT activity. Misra and Fridovich (1972) approach was used to calculate the activity of the liver's superoxide dismutase (SOD). The Action of superoxide dismutase could be calculated using the needed amount of protein to reduce epinephrine autooxidation by 50% for one minute. The catalase (CAT) activity was measured in nanomoles of H_2O_2 decomposed per minute per mg protein, according to Takahara *et al*. (1960).

Blood, serum sampling and analysis

After five fish are being used in the experiment, chosen at random from each of the tanks (15 per treatment) and anesthetized (clove powder, 200 mg L^{-1}) a 2 mL syringe to draw blood samples from the caudal vein. The serum was separated by centrifuging the samples spun at 4000 rpm for 5 min using non-heparinized tubes at 4°C. For the hematological indices experiment, Blood was drawn into heparinized tubes and allowed to coagulate for 40 min at room temperature. Leucocyte and erythrocyte count and hemoglobin concentrations were all calculated using whole blood samples. The total amount of red blood cells (RBCs) and white blood cells (WBCs) in the body (WBCs) were calculated using the Jain (1976) and Carrol procedure. According to Rook and Dennis (1985), The reduction of nitro blue tetrazolium was used to calculate the formation of superoxide ions by leukocytes (NBT, Sigma-Aldrich Chemical, USA). Using a Semi-auto Chemistry Analyzer, Erba Mannheim Mannheim kits, serum glucose, total protein, and albumin levels were calculated using these methods (Rayto RT-9200, GmbH, Germany). Albumin values were subtracted protein derived from whole serum to measure globulin. Divide albumin measurements by globulin values to get the albumin–globulin ratio.

Statistical analysis

The study used a complete randomized design. The mean and the data's standard error were computed. At a 95 percent confidence level, the results were subjected to a one-way analysis of variance using the Statistical Package for Social Sciences (SPSS, version 16). Duncan's multiple range test was used to measure the degree of significance between means.

Results

Nutrient and antinutrient profile of MLM

MLM's nutritional and antinutrient profile is given in Table 2. Phyto-chemical analysis of *M. bracteata* leaves showed that tannin content was 21.16 ± 0.43; phytate was 13.76 ± 0.15; alkaloid was 2.40 ± 0.01 and saponins was 13.37 ± 0.44.

Table 1: The experimental diets' proximate content and the formulation was given to *C. carpio* fingerlings for 60 days

Ingredient's composition (g kg^{-1})	Diets (Treatments)[1]			
	Control	MLM10	MLM20	MLM30
DSBM [2]	38	38	38	38
GNOC[3]	19.5	15	10.5	6
Wheat flour	5.3	9.8	14.2	18.7
DORB[4]	30	20	10	0
MLM[5]	0	10	20	30
Soybean oil & fish oil (1:1)	4.2	4.2	4.2	4.2
Vitamin-mineral mix[6]	1.2	1.2	1.2	1.2
Choline chloride	0.2	0.2	0.2	0.2
CMC[7]	1.5	1.5	1.5	1.5
BHT[8]	0.1	0.1	0.1	0.1
Proximate composition (dry matter basis)				
Dry matter (%)	90.33	90.87	91.07	90.86
Crude protein (%)	32.08	31.99	32.09	31.94
Ether extract (%)	6.05	6.02	6.04	6.02
Crude fiber (%)	7.14	7.25	7.34	7.74
Nitrogen free extract (%)	46.34	46.23	45.72	43.99
Total ash (%)	8.45	8.51	8.87	8.82
DE[9] (%)	368.54	367.54	365.19	358.44
P:E[10] (%)	87.04	87.03	87.87	89.11

The mean results of triplicates are used to calculate proximate composition.
1) Control = 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC 2) DSBM = De oiled soybean meal; (3) GNOC = Groundnut oil cake; (4) DORB De oiled rice bran; (5) MLM = *Mucuna bracteata* leaf meal (6) Composition of vitamin mineral mix (AGRUMIN 3503) (quantity kg^{-1}) Vitamin A, 7,00,000 IU; Vitamin D3, 7,00,000 IU; Vitamin E, 250 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 1 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 325mg; Fe, 1,500 mg; Zn, 6,000 mg; Cu,1,000 mg; Co,150 mg; Lysine, 10 g; Methionine, 10 g; Selenium, 125 mg; Vitamin C, 2,500 mg (7) CMC = Carboxymethyl cellulose; (8) BHT = Butylated hydroxytoluene (9) DE (Kcal 100 g^{-1}), Digestible energy = {(kcal 100 g^{-1}) = (%CP*4) + (%EE*9) + (%NFE*4)}; (10) P:E (mg protein $kcal^{-1}$ DE), Protein to energy ratio (mg protein $kcal^{-1}$ DE) = (%CP*1000)/DE (kcal 100 g^{-1})

Table 2: Proximate composition and antinutritional factors of MLM

Particulars	Mucuna leaf meal
Proximate composition (%)	
Dry matter	103.19 ± 0.13
CP	28.12 ± 0.18
CL	2.63 ± 0.05
CF	17.69 ± 0.62
NFE	44.79 ± 0.79
ASH	18.76 ± 0.45
Gross energy (kcal/100g)	370.61 ± 8.76
Antinutritional factor	(mg/100 g)
Total tannins	21.16 ± 0.43
Alkaloid	2.40 ± 0.01
Phytic acid	13.76 ± 0.15
Saponin	13.37 ± 0.44

Data represent mean ± S.E (n=6) MLM = *M. bracteata* leaf meal; C.F = crude fiber; CL = crude lipid; CP = crude protein; NFE = nitrogen-free extract; ASH = total ash content

Proximate composition of whole-body fish and experimental diets

Table 1 shows biochemical dietary composition in experiments. During the experiment, chemical constituents such as dry matter (90.33–91.07), crude protein (31.94–32.09), ether extract (6.02–6.05), crude fiber (7.14-7.74) ash content (8.45–8.87) and total carbohydrate (43.99-46.34) showed no significant ($P > 0.05$) alterations. Table 3 shows

Table 3: Proximate analysis of the whole body of several experimental groups of *C. carpio* fingerlings (%wet weight basis)

Treatment[1]	Moisture	Crude protein	Ether extract	Total carbohydrate	Total ash
Control	$73.85^{ab} \pm 0.83$	$16.01^{cd} \pm 0.08$	3.98 ± 0.17	3.72 ± 0.24	2.47 ± 0.15
LM15	$74.59^{ab} \pm 0.61$	$15.54^{bc} \pm 0.38$	3.83 ± 0.18	3.85 ± 0.28	2.23 ± 0.15
LM30	$75.85^{c} \pm 0.59$	$14.51^{a} \pm 0.31$	3.41 ± 0.21	3.95 ± 0.23	2.30 ± 0.13
CEE	$73.74^{ab} \pm 0.84$	$16.32^{cd} \pm 0.34$	4.12 ± 0.16	3.42 ± 0.22	2.44 ± 0.26

All data are presented as Mean S.E. (n=6). The difference between the mean values in the same column with different superscripts is significant ($P < 0.05$)
1 Control, 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC

Table 4: Protein metabolic enzyme activities in *C. carpio* fingerlings fed various experimental diets fish 60 days

Treatments[1]	Protein metabolic enzymes			
	AST[2]		ALT[3]	
	Liver	Muscle	Liver	Muscle
Control	1.28 ± 0.01	1.29 ± 0.11	$2.76^{b} \pm 0.34$	$2.54^{b} \pm 0.02$
MLM10	1.33 ± 0.09	1.36 ± 0.12	$2.45^{b} \pm 0.18$	$2.32^{b} \pm 0.02$
MLM20	1.49 ± 0.07	1.68 ± 0.14	$3.72^{a} \pm 0.26$	$3.17^{a} \pm 0.37$
MLM30	1.27 ± 0.03	1.28 ± 0.12	$2.11^{b} \pm 0.26$	$1.95^{b} \pm 0.01$

The data is shown as Mean Standard Error (n=6); the difference between same-column mean values with distinct superscripts is significant ($P < 0.05$)
1 Control, 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC
2 AST, Aspartate aminotransferase, the amount of oxaloacetate released is measured in nanomoles. min^{-1} mg^{-1} protein at 37°C; [3]ALT, Alanine aminotransferase, nanomoles of sodium pyruvate released are used to measure particular activity min^{-1} mg^{-1} protein at 37°C

Table 5: Hepatic antioxidant enzyme activities in *C. carpio* fingerlings fed various experimental diets fish 60 days

Treatments[1]	SOD[2]	CAT[3]
Control	$9.06^{c} \pm 0.09$	$26.18^{b} \pm 0.15$
MLM10	$9.09^{c} \pm 0.06$	$24.94^{a} \pm 0.01$
MLM20	$9.45^{b} \pm 0.06$	$31.93^{d} \pm 0.04$
MLM30	$13.38^{a} \pm 0.23$	$29.13^{c} \pm 0.04$

The data is shown as a mean SE (n=6); the difference in mean values in the same column with different superscripts is considerable ($P < 0.05$). 1Control, 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC
[2]SOD, Superoxide dismutase, Inhibition of epinephrine auto-oxidation by 50% is the measure of specific action mg^{-1} protein min^{-1}; [3]CAT, Catalase, nanomoles are used to measure particular activity H_2O_2 decomposed min^{-1} mg^{-1} protein

entire fish carcass composition fed varied experimental diets. During experiment, chemical constituents such as moisture (73.74–75.85) and crude protein (14.51–16.32) showed differences that are significant ($P < 0.05$). The crude lipid (3.41–4.12), ash content (2.23–2.47) and total carbohydrate (3.42-4.12) showed no significant ($p > 0.05$) shifts.

Protein metabolic enzyme and oxidative stress enzyme

The ALT activity of muscle and liver (Table 4) fluctuated considerably between dietary groups ($P < 0.05$). Between experimental groups, there was no significant difference in AST activity in muscle or liver ($P > 0.05$). Hepatic SOD and catalase behavior (Table 5) a significant difference between the experimental group ($P < 0.05$). Hepatic SOD activity was higher in MLM30% fed fish ($P < 0.05$) compared in control fish, while fish given only 20% dietary MLM had considerably higher liver catalase (CAT) activity compared to the control group ($P < 0.05$).

Haemato-immunological profile

MLM leaf meal had a major impact on the overall RBC count. A rise in RBC count was observed as the MLM level increased (Table 6). The count of RBCs and WBCs in the control group and The MLM10% groups were statistically comparable ($P > 0.05$). The NBT values differ significantly ($P > 0.05$) among the treatments. When MLM was used, the NBT value increased dramatically in comparison to a control group. The treatment with MLM30 had a higher NBT value.

Serum proteins

Table 7 shows albumin, globulin and total protein levels in the blood, for various experimental groups. The control group had the highest albumin blood albumin-globulin ratio and concentration, which was not statistically different ($P > 0.05$) from other groups given leaf meal but was higher in comparison to other groups. The MLM10 % group had the highest serum globulin levels; this was statistically different from the other groups ($P > 0.05$). The serum glucose levels (Table 7) a significant difference among the treatments ($P > 0.05$). MLM participation in 10% and 20% resulted in a substantial reduction in serum glucose levels as in comparison to a control group, while participation in 30% resulted in an increase in serum glucose level but the increase was not significant.

Discussion

This the purpose of the research was to see if raw *Mucuna*

Table 6: Hematological and haemato-immunological parameters of *C. carpio* for 60 days, fingerlings were fed various experimental diets

Treatments[1]	Hemoglobin (g dL^{-1})	TEC[2] ($x10^6$ cmm^{-1})	TLC[3] ($\times10^4$ cmm^{-1})	NBT[4] (OD 620 nm)
Control	$7.25^{c} \pm 0.05$	$2.00^{b} \pm 0.03$	$14.05^{b} \pm 0.43$	$0.55^{b} \pm 0.01$
MLM10	$8.08^{b} \pm 0.12$	$1.96^{b} \pm 0.02$	$14.03^{b} \pm 0.08$	$0.58^{b} \pm 0.02$
MLM20	$8.93^{a} \pm 0.04$	$2.21^{a} \pm 0.01$	$12.49^{c} \pm 0.32$	$0.65^{a} \pm 0.01$
MLM30	$7.46^{c} \pm 0.04$	$1.89^{c} \pm 0.02$	$15.69^{b} \pm 0.43$	$0.68^{a} \pm 0.01$

The data is shown SE as Mean (n=6); the difference in mean values in the same column with different superscripts is considerable ($P < 0.05$)

[1]Control, 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC [2]TEC, Total erythrocyte count; [3]TLC, Total leucocyte count; [4]NBT, Nitoblue tetrazolium

Table 7: Haemato-biochemical characteristics in fingerlings of *C. carpio* fed various experimental diets for 60 days

Treatments[1]	Serum TP[2] (g dL^{-1})	Serum Alb[3] (g dL^{-1})	Serum Glob[4] (g dL^{-1})	Serum A:G[5]	Serum Glu[6] (mg dL^{-1})
Control	3.03 ± 0.24	1.37 ± 0.23	$1.57^{c} \pm 0.01$	0.87 ± 0.12	$82.15^{a} \pm 0.33$
MLM10	2.93 ± 0.07	1.12 ± 0.02	$1.94^{b} \pm 0.06$	0.57 ± 0.01	$80.43^{b} \pm 0.54$
MLM20	3.01 ± 0.04	1.16 ± 0.03	$1.54^{c} \pm 0.03$	0.75 ± 0.02	$79.42^{b} \pm 0.31$
MLM30	3.08 ± 0.13	1.20 ± 0.07	$1.74^{a} \pm 0.05$	0.68 ± 0.02	$83.09^{a} \pm 0.37$

The data is shown as Mean Standard Error (n=6); the difference between mean values in the same column with different superscripts is significant ($P < 0.05$). 1Control, 30% DORB and 0% MLM; MLM10, 10% MLM in replacement of 33.3% DORB and 23.4% GNOC; MLM20, 20% MLM in replacement of 66.6% DORB and 46.8% GNOC; MLM30, 30% MLM in replacement of 100% DORB and 70.2% GNOC

[2]TP, Total protein; [3]Alb, Albumin; [4]Glob, Globulin; [5]A:G, Albumin to globulin ratio; [6]Glu, Glucose

DORB could be replaced in the diet with leaf meal of common carp. However, there have been no studies on *M. bracteata* leaf meal has been used as a DORB alternative in fish diets. *M. bracteata* leaf meal is used in fish diets has never been documented., but there is information on other leaf meals. Leaf meals are inexpensive, easily accessible, and have no direct competitors in the livestock feed industry. Leaf meals can be utilized in the *C. carpio* diet as an alternate aquafeed component, several prior studies have found (Meshram *et al.* 2018; Anand *et al.* 2019; Ahmad *et al.* 2019; Maiti *et al.* 2019; Jayant *et al.* 2020; Rani *et al.* 2020; Sahoo *et al.* 2020). The major challenge in limiting the use of alternative feed sources of plant origin is its fish acceptability, which is typically linked to the diet's palatability (Rodriguez and Perston 1996). The majority of tannins, oxalates, phytic acid, alkaloids and other anti-nutritional substances and other anti-nutritional factors can be found in plant substances (NRC 1993), which obstruct nutrition use and have detrimental consequences for animal growth and other physiological functions, including fish. Total tannins, phytic acid, saponins, and total alkaloids were discovered in MLM. For the anti-nutrient content of MLM, there are no quantitative reports available. Anand *et al*. (2019) reported that *C. carpio* fed a 15% raw Sesbania leaf meal-based diet had worse development efficiency, most likely due to increased saponin levels. Green pea meal could be used to substitute 10% of fish meal in barramundi and Lates calcarifer feed without affecting growth or nutrient utilization (Ganzon-Naret 2013). Puycha *et al*. (2017) found that higher inclusion of moringa, Swai catfish were fed 150 to 200 grams of *Moringa oleifera* leaf meal per kilogram of body weight, *Pangasius bocourti*, possibly due to its high phytate content (0.4%). Dietary tannin and phytic acid concentrations of 0.5-2.0% and 0.5-0.6%, respectively, were found to reduce *C. carpio* growth performance (Hossain and Jauncey 1990). The experimental diets using mucuna seed meal demonstrated a high acceptability and no essential amino acid (EAA) deficits (Siddhuraju and Becker 2001). Sweet potato leaf meal, raw (Meshram *et al.* 2018) and *Hygrophila spinosa* leaf meal (Maiti *et al.* 2019) were found to DORB should be fully replaced (30% incorporation) within the feed of *Labeo rohita*.

Proximate composition of the *M. bracteata* leaves indicated that the leaves of *M. bracteata* are relatively rich in crude protein (28.12 ± 0.18). Janardhanan and Lakshmanan (1985) discovered that the seeds of *M. pruriens var. utilize* to have a high crude protein content (26.25–29.6%). According to Jambunathan and Singh (1980), the crude protein value of pulses, pigeon pea, black gram, red gram, chickpea and green gram are often consumed, appears to be lower than mucuna. The protein level, in particular, tends to compare favorably to recorded levels of other traditional protein sources, especially those of plant origin (Nwokolo and Oji 1985; Fasuyi *et al*. 2008). Mucuna meal-based diets resulted in the highest carcass moisture and crude protein levels and contain the least amount of lipids and energy. Surprisingly, the difference was insignificant in carcass moisture, protein, ash, or calorie value between the treatment diets. This may be due to the experimental diets being isonitrogenous and isolipidic (Yengkokpam *et al.* 2013; Fawole *et al.* 2016; Garg *et al.* 2019).

When fish are stressed and have an energy shortfall, existing amino acids are converted to a different amino acid by transaminases, which are subsequently reduced for the generation of energy from keto acid gluconeogenesis (Silva and Anderson 1995; Chatterjee *et al*. 2006). As a result, the availability of nonproteins, the need for amino acids in energy synthesis can be reduced by using alternative energy sources. In this way, ALT and AST help fish growth by serving energy metabolism as a link between protein, carbohydrates, and fats (Shamna *et al.* 2015). As a result, the activity of the AST and ALT enzymes can be useful markers in terms of amino acid metabolism in fish (Lin and Luo

2011; Jiang *et al.* 2015). Higher ALT and AST activities in the MLM20 group in this study suggested that fish could make nonprotein energy, particularly carbohydrate, available at an optimal level, thus sparing amino acids facilitate the production of body proteins, resulting in increased fish development.

When tissue free radicals are abnormally strong, animals suffer from oxidative stress (Sies and Groot 1992). Antioxidant defense mechanisms in all organisms protect them from ROS-mediated cellular and nutrient damage, with CAT and SOD playing a key role in lowering ROS levels in tissues. Catalase and peroxidase are enzymes that convert superoxide ions to water, hence an increase in their activity is thought to boost antioxidant defenses (Dawood *et al*. 2017b; Abdel-Tawwab and Monier 2018; Hoseinifar *et al.* 2018; Doan *et al.* 2019). SOD converts the poisonous superoxide anions to hydrogen peroxides, which are then degraded by CAT into oxygen and water. SOD and CAT movements are thus increased during oxidative stress to neutralize ROS. As a result, when there is oxidative stress, SOD and CAT activity both rise. In this work, higher SOD and CAT levels in MLM groups that have been fed demonstrated oxidative stress caused by ANF in fish.

Hematological parameters in fish have been demonstrated to be improved by extracts from plants (Reverter *et al*. 2014; Dawood *et al*. 2017a). As the amount of *Mucuna* leaf meal as a dietary supplement increased, the number of red blood cells increased. The rise in WBC in common carp fed MLM enriched meals was found, particularly in this study, the MLM20 supplemented diet was used, which could be due to MLM's immunostimulatory properties. WBC numbers have phagocytic activity as well as biomarkers for immunological function. According to Valenzuela-Grijalva *et al*. (2017), immunostimulatory activity in animals is one of the biological effects of phytogenic feed additives. Between the control and treatment groups, there was a substantial difference in RBC, Hb and WBC ($P > 0.05$). In this analysis, indices like RBC and Hb in *C. carpio* through the different dietary treatments indicate that the MLM diet supports or does not obstruct regular functioning hemopoiesis processes. The higher RBC levels noticed in fish-fed individuals' MLM20 diets suggest that the dietary protein content is higher and that MLM20 improved blood quality. Increased RBC values were related to high-quality dietary protein and disease-free livestock, according to Hackbarth *et al*. (1983). The measurement of oxidative radical generation is critical for evaluating the stimulation of cellular defense in fish. Some immune cells, such as neutrophils and macrophages, use oxygen during this process and produce reactive oxygen species (ROS), which are harmful to pathogenic germs (Srivastava and Pandey 2015). In a reduction reaction, these ROS interact with nitro blue tetrazolium (NBT), showing whether or not oxidative radical generation has risen.

Conclusion

Finally, the findings imply that feeding common carp a diet containing 20% MLM for 60 days may be sufficient to increase immunological parameters, protein metabolic enzymes, and antioxidant status. As a result, Mucuna leaf meal can be used to substitute regular rice bran that has been de-oiled in common carp feed. Mucuna leaf meal offers potential as a rice bran replacer, according to the findings of this study. The nutritional quality of *M. bracteata* leaf meal, on the other hand, was adequate, resulting in metabolic and antioxidant enzyme activities. However, the inclusion of MLM at 20% exhibited higher better physio-metabolic responses in fish than DORB. As a result, more to analyze and understand the effects of varying quantities of dietary mucuna leaf meal on a variety of finfish species, more research is needed.

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Author Contributions

Ashutosh Dharmendra Deo and Narottam Prasad Sahu planned experiments, Tincy Varghese and Hafeef Roshan interpreted the results, Hafeef Roshan made the review and genuinely broke down the information and made outlines.

Conflict of Interest

The authors do not have any conflict of interest to declare.

Data Availability

The appropriate author will be able to provide data upon request

Ethical Approval

The institutional ethical committee gave its approval to all of the ethical rules that were followed.

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