



Dietary optimisation of black gram (*Vigna mungo*) leaf meal as substitute for deoiled rice bran in the diet of *Labeo rohita* (Hamilton, 1822) fingerlings

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ABSTRACT

The study aimed to evaluate the potential of black gram (*Vigna mungo*) leaf meal (BGLM) as a substitute for deoiled rice bran (DORB) in the diet of *Labeo rohita* (Hamilton, 1822) fingerlings. Four isonitrogenous (30% CP) and isocaloric (420 Kcal 100 g⁻¹ gross energy) experimental diets were prepared to conduct the study with different inclusion levels of BGLM viz., control (0 BGLM; 30% DORB), LM10 (10% BGLM, 20% DORB), LM20 (20% BGLM, 10% DORB) and LM30 (30% BGLM, 0% DORB). One hundred fortyfour fishes with an initial mean weight of 3.52±0.04 g were arbitrarily assigned to the indoor experimental tubs in triplicates for the 60 days experiment. Fish were fed with experimental diets to satiation twice daily. Percent weight gain and specific growth rate (SGR) were significantly ($p<0.05$) higher in LM20 group than the other treatment groups (LM10 and LM30) showing no significant ($p>0.05$) difference with the control group. Among the digestive and metabolic enzyme activities, lipase, hepatic aspartate amino transferase (AST) and alanine amino transferase (ALT) levels were found to be higher in LM20 group compared to the other groups. The study concluded that BGLM can completely replace DORB in the diet of *L. rohita*. However, the best growth and metabolic performance can be achieved with the inclusion of 20% leaf meal.

Keywords: Anti-nutritional factor, Deoiled rice bran, *Labeo rohita*, Leaf meal, *Vigna mungo*

Introduction

Vertical expansion of aquaculture has become the need of the hour to fulfill the huge gap between supply and demand of fish. Intensive aquaculture requires quality feed unlike the extensive one. Providing good quality feed depends upon the quality of ingredients used to prepare the feed. Due to competition with other animal feed production sectors, the price of most of the traditional ingredients has increased sharply in recent years forcing researchers to explore non-conventional ingredients. Among the conventional ingredients, oilcakes and rice bran are commonly incorporated in feed for both fish and poultry. Farmers of the Asian subcontinent mostly use deoiled rice bran (DORB) or rice bran in fish feed. Sometimes farmers feed the fishes with rice bran in wet mass or dry form without adding other ingredients. Besides use in animal feed, rice bran is also processed for human consumption. Hence, it has become a necessity to find an alternative to rice bran or deoiled rice bran in preparation of aquafeeds. As an alternative to conventional ingredients, leaf meal can be one of the potential and cheapest ingredient sources for aquafeed (Adewolu, 2008). Many studies have been carried out on leaf meal utilisation in fish diet by various authors such as duck weed in *Labeo rohita* (Bairagi *et al.*,

2002); *Moringa oleifera* leaf meal in *Oreochromis niloticus* (Richter *et al.*, 2003); *Garcinia gummi-gutta* plant extract in *Pangasianodon hypophthalmus* (Sheeno and Sahu, 2006); *Ipomoea batatas* leaf meal in *Tilapia zilli* (Adewolu, 2008); *Leucaena leucocephala* leaf meal in *Clarias gariepinus* (Amisah *et al.*, 2009); *Eichhornia crassipes* leaf meal in *L. rohita* (Saha and Ray, 2011); mulberry leaf meal in *L. rohita* (Mondal *et al.*, 2012); fermented mulberry leaf meal in *L. rohita* (Kaviraj *et al.*, 2013); sweet potato leaf meal in *L. rohita* (Meshram *et al.*, 2018; Ahmad *et al.*, 2019) and *H. spinosa* leaf meal in *L. rohita* (Maiti *et al.*, 2019).

There are different types of wastes produced from crop production which are not used for human consumption. These agricultural wastes are called trash or crop residues. These residues like corn stalks, corn cobs, wheat straw and other leftover grain products are gaining economic importance. These wastes are traditionally used to feed the animals in many parts of the country. The harvest and post-harvest losses are very high in Indian agriculture system. For example, after harvesting, farmers usually throw the whole bengal gram plant containing leaves and stems, which are rich in nutrients. Thus, leaves and stems, otherwise dumped as waste, can be evaluated for inclusion

in aquafeed as an alternative to DORB. The Fabaceae family consists of several gram varieties in which black gram (*Vigna mungo*), popularly known as urad dal, is one of the most important crops grown across the tropical and sub-tropical countries. This crop can withstand adverse climatic conditions and improve soil fertility.

The crude protein content of black gram foliage is around 15.5% (Dey *et al.*, 2017), while the major restriction in utilising these leaves in aquafeed is the presence of anti-nutritional factors like phytic acid, saponin and polyphenol (Kataria *et al.*, 1989). Hence, the present study aimed at selecting the best possible inclusion level of black gram leaf meal (BGLM), substituting DORB in the diet of *L. rohita* fingerlings.

Materials and methods

Preparation of black gram leaf meal (BGLM)

After harvesting of black gram seeds, the leaves were collected from the farmers' field; packed in neat polythene bags and transported to the laboratory of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Mumbai, India. The leaves were dried at 60°C in a hot air oven and pulverised to a fine powder for further use.

Experimental animal

The experimental fish, *L. rohita* fingerlings were procured from Aarey Fish Farm, Goregaon (E), Mumbai

and transported to the wet laboratory in oxygen filled polyethylene bags. They were carefully transferred to a cement tank (2000 l) for acclimatisation for 15 days while they were fed a control diet having 30% crude protein up to satiation.

Diet formulation and preparation

Four isonitrogenous (30% CP) and isocaloric diets [420 kcal 100 g⁻¹ gross energy (GE)] were formulated with different levels of black gram leaf meal (BGLM) to replace deoiled rice bran (DORB) in the diet viz. control (0% BGLM; 30% DORB), LM10 (10% BGLM; 20% DORB), LM20 (20% BGLM; 10% DORB) and LM30 (30% BGLM; 0% DORB). Feed ingredients were weighed (Table 1) and mixed to form a dough with the addition of required quantity of water. The dough was put in an aluminium container and placed in a pressure cooker for cooking or steaming. Steaming was done for 15 to 30 min after which it was cooled for some time. After cooling, the dough was mixed properly with oil and vitamin-mineral mixture and then through a pelletiser, and uniform sized pellets were prepared using a die (2 mm). Pellets were spread on a paper sheet, air dried and stored at -20°C for further use.

Experimental set up

All fishes were starved for 24 h prior to commencement of the experiment. The feeding trial was

Table 1. Feed formulation and composition of different experimental diets

Ingredients	C	LM 10	LM 20	LM 30
DSBM	30	29.75	29	28.3
GNOC	25.93	25.93	25.93	25.93
Wheat flour	6.75	6.75	7.5	8.2
DORB	30	20	10	0
BG LM	0	10	20	30
Soybean oil	2	2	2	2
Cod liver oil	2	2	2	2
Vit-min mix	2	2	2	2
Choline chloride	0.3	0.3	0.3	0.3
CMC	1	1.25	1.25	1.25
BHT	0.02	0.02	0.02	0.02
Proximate composition (% dry matter)				
Crude protein	31.32±0.37	30.53±0.2	31.06±0.41	31.18±0.4
Ether extract	5.85±0.51	5.46±0.03	5.33±0.05	5.97±0.15
Nitrogen-free extract	48.53±0.91	49.37±0.19	45.59±0.38	43.79±0.25
Crude fibre	6.51±0.02	7.17±0.03	7.28±0.04	7.53±0.02
Total ash	7.8±0.01	7.37±0.06	10.5±0.12	11.9±0.03
Gross energy(kcal g ⁻¹)	4.42±0.00	4.48±0.01	4.28±0.00	4.08±0.02

Quantity of ingredients are expressed in g 100 g⁻¹; DSBM - Defatted soybean meal; GNOC - Groundnut oil cake; DORB - Deoiled rice bran; BGLM - Black gram leaf meal; CMC - Carboxymethyl cellulose; BHT - Butylated Hydroxytoluene; Vit-min mix-Vitamin mix and mineral mixture. Composition of vitamin mineral mix (PREMIX PLUS) (quantity kg⁻¹): Vitamin A - 55,00,000 IU; Vitamin D₃ - 11,00,000 IU; Vitamin B₂ - 2,000 mg; Vitamin E - 750 mg; Vitamin K - 1,000 mg; Vitamin B₆ - 1,000 mg; Vitamin B₁₂ - 6 µg; Calcium pantothenate - 2,500 mg; Nicotinamide - 10 g; Choline chloride - 150 g; Mn - 27,000 mg; I - 1,000 mg; Fe - 7,500 mg; Zn - 5,000 mg; Cu - 2,000 mg; Co - 450; L - lysine - 10 g; DL - Methionine - 10 g; Selenium - 125 mg; Vitamin C - 2500 mg. Nitrogen free extract = [100- (%Crude protein + %Crude lipid + %Crude fibre + %Ash)]

conducted in rectangular plastic tubs (57×36×47 cm; 96 l capacity) covered with perforated lids for 60 days in the wet laboratory of ICAR-CIFE, Mumbai. Round the clock aeration was provided through the experimental period in all experimental units. One hundred fortyfour fingerlings with an average weight of 3.52 ± 0.04 g were randomly allocated to four experimental groups in triplicate following a completely randomised design. The fishes were fed to satiation twice daily (09.00 and 16.00 hrs) for 60 days. Faecal matter was siphoned out everyday and a constant volume of water (75 l) in each tub was maintained throughout the experimental period with round the clock aeration. The water quality parameters viz., temperature, pH, dissolved oxygen and ammonia were assessed following APHA (1998) and found within the ideal range for Indian major carps (temperature, 28-30°C; pH, 7.3-8.4; dissolved oxygen, 6-7.5 mg l⁻¹; ammonia, 0.04-0.11 mg l⁻¹).

Evaluation of nutrients and anti-nutritional factor

Proximate analysis of BGLM, experimental diets and experimental fish was done following AOAC (1995). At the end of the feeding trial, three fish each were sampled from each replicate to analyse the body proximate composition. Moisture content was estimated by drying the samples at 105°C in an oven until a fixed weight was achieved. Crude protein (CP) was determined using the Kjeldahl digestion method (N×6.25). Ether extract (EE) was estimated by soxhlet-extraction method. Total ash (TA) content was determined by combustion of the sample in a muffle furnace at 550°C for 6 h. Crude fibre (CF) was determined using the acid/base digestion process. Nitrogen free extract (NFE) was calculated by taking the sum of values for crude protein, crude lipid, crude fibre and moisture and then subtracting this from 100 (Maynard *et al.*, 1979). In case of the fish sample, CF was not determined; rather total carbohydrate (TC) was calculated by deducting the sum of CP%, EE% and TA% from 100.

The amino acid contents of BGLM were analysed by automated pre-column derivatisation method through HPLC using o-phthalaldehyde reagent (Fekkes *et al.*, 1995). In short, 10 µl of the digested sample was mixed with 60 µl borate buffer and 10 µl of OPA reagent and vortexed. From this mixture, 50 µl sample was injected in the HPLC column and the chromatogram obtained; and calibrated along with the standards and used for calculation of individual amino acids.

The anti-nutritional factors and other active compounds like flavonoids present in BGLM were quantified following different methods. The phytic acid in the leaf extract was determined according to the spectrophotometric method described by Gao *et al.* (2007) with some modifications. Exactly 0.1 ml of sample extract

was diluted to 3 ml with distilled water and then mixed with 1ml of Wade reagent (0.03% FeCl₃·6H₂O + 0.3% sulphosalicylic acid). The mixture was centrifuged for 10 min at 5000 rpm after keeping for 30 min and then the supernatant was taken for measuring absorbance at 500 nm against distilled water. The phytic acid content was calculated from the standard calibration curve.

For the determination of total tannin, spectrophotometric method as described by Makkar *et al.* (2007) was followed. The reagent used was Folin-Ciocalteu reagent and the results were expressed as tannic acid equivalents. The total tannin content was calculated from the calibration curve of the tannic acid standard.

Oxalate content was determined through a titration method using potassium permanganate as titrant as described by Nwosu (2011). Alkaloid content was estimated by gravimetric method (Haborne, 1973). Five gram of leaf sample was added into 50 ml of 10% acetic acid solution with ethanol. The sample was shaken well and allowed to stand for 4 h and filtered. The filtrate was then evaporated to one-quarter of its original volume on a hot plate. To precipitate alkaloids, concentrated ammonium hydroxide was added drop by drop until it got precipitated. The precipitate was filtered on a pre-weighed filter paper, dried upto constant weight and final weight taken. The alkaloid content was estimated by taking the difference in weight and expressed as percentage weight of the sample.

Total flavonoid content was determined following the method of Park *et al.* (2008). In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO₂ (0.5 M) and 0.15 ml of AlCl₃·6H₂O (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1M) was added. The solution was mixed appropriately and the absorbance was recorded against the reagent blank at 506 nm. Flavonoid content was estimated from the standard graph made with rutin, as per the same protocol followed for sample analysis.

In vitro relative protein digestibility

In vitro protein digestibility (IVPD) of BGLM and diets were analysed according to the method of Ali *et al.* (2009). Prior to the feeding trial, ten acclimatised fish (2.95 ± 0.34 g) were taken from the stock and dissected out to collect the fish gut. Then, dissected fish guts were homogenised with distilled water (1:10 w/v) for extraction of the gut enzymes. The enzymatic supernatants were collected after centrifugation of the tissue homogenate at 12000 rpm for 15 min at 4°C. The enzyme extract was kept at -20°C until use. An equivalent amount of finely ground and sieved ($\leq 40\mu$) sample that provided 160 mg of CP was taken for digestibility study. Samples were diluted with distilled water to get 8 mg CP ml⁻¹. Finally,

the blended enzymes were added with the sample and the pH was adjusted to 8. The drop in pH was observed at every minute for 10 min. Casein was used as the reference protein to assess the digestibility.

Relative protein digestibility (RPD %) = $(-\Delta\text{pH of ingredients} / -\Delta\text{pH of casein}) \times 100$

Evaluation of growth performance

Growth parameters *viz.*, weight gain percentage, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were estimated using the following formulae:

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

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}}{\text{Number of days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g Dry weight)}}{\text{Body weight gain (g Wet weight)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Net weight gain}}{\text{Protein fed}}$$

Preparation of tissue homogenate

At the end of experiment, three fish from each experimental tub were anaesthetised with clove oil (50 $\mu\text{l l}^{-1}$). The fish tissues *viz.*, muscle, liver and intestine were dissected out under ice-chilled condition and tissue homogenate (5%) was prepared in chilled sucrose solution (0.25 M) using a mechanical tissue homogeniser (MICCRA D-9, Germany). Supernatants were collected after centrifuging the homogenate at 5000 rpm for 10 min at 4°C. Collected tissue homogenates were stored at -20°C to perform the enzyme assays.

Digestive enzyme assays

Protease activity was estimated by the casein digestion method (Drapeau, 1974). One unit of protease enzyme specific activity was defined as the amount of enzyme required to release acid soluble fragments equal to $\Delta 0.001$ at 280 nm per min at 37°C and pH 7.8. Amylase activity was assessed as the reducing sugars produced from the action of glucoamylase and α -amylase on carbohydrate using di-nitro-salicylic-acid (DNS) method (Rick and Stegbauer, 1974). Specific amylase activity was expressed as a mole of maltose released from starch per min at 37°C. The lipase activity was determined by the titrimetric method of Cherry and Crandell (1932) which is based on the measurement of fatty acids released by

the enzymatic hydrolysis of triglycerides present in a stabilised emulsion of olive oil.

Metabolic enzyme assays

Two important enzymes of protein metabolism, *i.e.* aspartate amino transferase (AST) and alanine amino transferase (ALT) were assayed in this study. AST activity from different tissues of *L. rohita* was assayed following the spectrophotometric method by Wootton (1964). The substrates used comprised DL-aspartic acid and α -ketoglutarate dissolved in phosphate buffer (pH 7.4). AST activity was expressed in terms of nano moles of oxaloacetate formed per mg protein per min at 37°C. ALT activity was estimated following the same procedure with one exception in substrate composition *i.e.* DL-alanine instead of aspartic acid. The ALT activity was expressed in terms of nanomoles pyruvate formed per mg protein per min at 37°C.

For studying carbohydrate utilisation and metabolism, two enzymes *viz.*, lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) assays were done. The LDH activity was assayed by the method of Wrobleiuski and Laude (1955). The specific enzyme activity was expressed as unit $\text{mg protein}^{-1} \text{ min}^{-1}$ at 25°C, where 1 unit was equal to $\Delta 0.01 \text{ OD min}^{-1}$. Malate dehydrogenase (MDH) activity was assayed by the method of Ochoa (1955) and the specific enzyme activity was expressed in unit $\text{mg protein}^{-1} \text{ min}^{-1}$ at 25°C where 1 unit was equal to $\Delta 0.01 \text{ OD min}^{-1}$.

Statistical analysis of data

Statistical significance of different enzyme activities was analysed using one-way analysis of variance (ANOVA) using SPSS 22 for Windows. Duncan's multiple range test was used for *post-hoc* comparison of means ($p < 0.05$) between different treatments. The assumption of homogeneity of variance was tested by Levene's test. Data presented in the text and tables are means $\pm$ standard error and statistical significance for all statistical tests was set at $p < 0.05$.

Results and discussion

Potential of a feed stuff can be evaluated by its chemical composition. The present study revealed that BGLM contain 17.92% crude protein, 3.88% crude lipid, 21.04% crude fibre and 21.98% total ash (Table 2). These values were slightly higher than the reported proximate composition values in *V. mungo* foliage by Dey *et al.* (2017). These differences may be due to soil type, harvesting time, nature of the sample, local varieties and processing methods. The leaf meal is rich in lysine, methionine, glycine and histidine (protein bound amino acid expressed in g per dietary protein), whereas aspartic

acid, serine and glutamine are present in very low quantity in BGLM (Table 2). The amino acid profile of BGLM showed that a good quantity of essential amino acids (protein-bound amino acid) are present in leaf protein. There is no published report on the amino acid composition of black gram leaf meal to date. The leaf meal is rich in lysine and methionine which has enhanced growth and improved the health status of fishes.

Digestibility and palatability are the common problems encountered due to the presence of anti-metabolites along with high fibre content of leaf meal. Tannins, phytic acid, total oxalate, alkaloids and total flavonoids were found to be present in BGLM (Table 2). However, presence of these anti-nutrients did not affect the palatability of the diets and overall growth performance in fishes. This may be due to very less amount of anti-nutrients present in black gram leaf meal. Digestibility is one of the

most crucial factors in evaluating the suitability of feed ingredients for a target fish species. In the present study, the *in vitro* protein digestibility of BGLM was found to be 24.76%, which was in agreement with *in vitro* apparent protein digestibility of rice bran (29.95%) reported in *C. gariepinus* (Udo and Umoren, 2011). The *in vitro* relative protein digestibility (RPD) of experimental diets was within the range of 72 to 84% (Table 3). Similar values were reported by Adewolu *et al.* (2008) who found RPD% of feed in the range of 75-80% in case of *Tilapia zilli* fingerlings fed with sweet potato leaf meal based diet.

The weight gain% was significantly different among various treatment groups (Table 4). The 20% BGLM (LM20) fed group showed a significantly ($p < 0.05$) higher weight gain% compared to other leaf meal fed groups but similar to the control group. Other treatment groups (LM10 and LM30) also did not vary significantly

Table 2. Proximate, amino acid composition and anti-nutritional profile of black gram leaf meal

Composition	BGLM (<i>V. mungo</i>)	
Proximate composition		
Moisture	10.37±0.02	
Crude protein	17.92±0.50	
Ether extract	3.88±0.07	
Nitrogen free extract	35.20±0.77	
Crude fibre	21.04±0.87	
Total ash	21.98±0.19	
Amino acid composition (g dietary protein ⁻¹)	Protein bound	Free amino acid
Aspartic acid	0.357	3.11
Glutamine	0.136	2.108
Serine	0.527	3.757
Histidine	4.947	5.814
Glycine	3.162	3.451
Threonine	2.907	5.814
Arginine	3.587	5.78
Alanine	3.519	1.819
Tyrosine	1.989	3.978
Methionine	3.128	9.571
Valine	2.584	4.59
Phenyl alanine	3.468	3.502
Isoleucine	1.853	2.312
Leucine	4.76	4.046
Lysine	3.451	9.095
Antinutritional profile		
Total tannin ¹	0.36±0.02	
Phytic acid ²	0.06±0.03	
Oxalate ¹	0.005±0.02	
Total flavonoid ³	2.97±0.05	
Alkaloid ¹	0.31±0.02	

Data expressed as mean±SE.

¹Total tannin, oxalate and alkaloid expressed in g 100 g⁻¹;

²Phytic acid expressed in g kg⁻¹;

³Total flavonoid content expressed as µg rutin equivalent per mg.

Table 3. *In vitro* relative protein digestibility of diets and black gram leaf meal (BGLM)

Time	Casein	BGLM	C	T1	T2	T3
ΔpH per 10 min	1.05	0.26	0.82	0.76	0.89	0.85
RPD (%)	100	24.76	78.09	72.38	84.76	80.95

Table 4. Growth parameters of the different experimental groups fed with the experimental diets for assessment of inclusion level of black gram meal

Treatments	IBW	FBW	Weight gain %	SGR	FCR	PER
C	3.50±0.05	6.55 ^{ab} ±0.31	87.14 ^{ab} ±9.02	1.04 ^{ab} ±0.08	2.29±0.34	1.52±0.23
LM-10	3.55±0.02	6.29 ^b ±0.19	77.28 ^b ±4.83	0.95 ^b ±0.05	2.35±0.15	1.42±0.09
LM-20	3.51±0.02	7.30 ^a ±0.31	107.98 ^a ±10.2	1.22 ^a ±0.08	1.78±0.09	1.88±0.09
LM-30	3.50±0.03	5.93 ^b ±0.12	69.63 ^b ±4.74	0.88 ^b ±0.05	2.61±0.18	1.29±0.09
p-Value	0.359	0.024	0.034	0.031	0.119	0.080

IBW - Initial body weight; FBW - Final body weight; SGR - Specific growth rate; FCR - Feed conversion ratio; PER - Protein efficiency ratio.

All values are expressed as mean±SE, n=3.

Mean values in the same column with different superscript differ significantly (p<0.05).

(p>0.05) from the control group. Specific growth rate (SGR) was also found to be higher in the LM20 group compared to LM10 and LM30 groups. FCR and PER values showed no significant difference (p>0.05) among the treatments. Available information on other leaf meals revealed that *Leucaena leucocephala* leaf meal can be incorporated at an inclusion level of 30% in the diets of *C. gariepinus*, but it is efficacious and cost-effective at 20% inclusion level which had the highest growth (Amisah *et al.*, 2009). Similarly, Nsofor *et al.* (2012) reported that *M. olifera* leaf meal could be included in diet upto 30% but 20% inclusion registered the highest growth. Afuang *et al.* (2003) reported that solvent-extracted *M. olifera* leaf meal could replace 30% of fish meal from *O. niloticus* diet. However, in the present study, the FCR and PER showed no significant difference among the groups. Though 30% inclusion level of duckweed leaf meal showed similar growth with control in tilapia, there was no significant difference (p>0.05) in FCR and PER among various treatments (Fasakin *et al.*, 1999).

The nutritional quality and utilisation of BGLM can be assessed from the body composition of *L. rohita* fingerlings (Table 5). Proximate composition of the whole body differed significantly (p<0.05) among the experimental groups. Fish fed with 30% BGLM showed

higher moisture content compared to all other groups, whereas lower inclusion levels did not show any variation from control. Moreover, the crude protein content was significantly lower in 30% BGLM fed groups compared to control indicating that higher inclusion level of BGLM significantly affects moisture and crude protein content of fish. A significantly higher level of crude protein in 20% BGLM fed group indicate higher protein accretion and growth at this inclusion level. A similar result was also reported by Bello and Nzeh (2013), who observed higher moisture content and lower protein level in the carcass of *C. gariepinus* fed with high inclusion level of *M. olifera* leaf meal. A lower lipid level was observed in LM30 groups, whereas control, LM10 and LM20 groups showed significantly higher lipid content compared to LM30 group. Similarly, Odedeyi *et al.* (2014) also reported a lower carcass lipid and protein content in *C. gariepinus* fed with 30% raw moringa leaf meal. Total ash content was found to be higher in LM10 and LM30 groups and a lower total carbohydrate content was observed in the LM20 group. Bello and Nzeh (2013) also reported increased ash content with increasing *M. olifera* leaf meal level in *C. gariepinus*.

Digestive enzymes such as protease, amylase and lipase are the enzymes which help in breaking down macro

Table 5. Carcass composition of *L. rohita* fingerlings in different experimental groups (% wet weight basis)

Treatments	Moisture	CP	TL	TA	TC
C	76.16 ^b ±0.09	13.07 ^b ±0.09	3.91 ^b ±0.06	2.78 ^b ±0.02	4.08 ^b ±0.19
LM-10	75.40 ^c ±0.19	12.84 ^{bc} ±0.12	4.29 ^a ±0.08	2.96 ^a ±0.04	4.71 ^a ±0.09
LM-20	75.86 ^b ±0.15	13.64 ^a ±0.09	4.23 ^a ±0.03	2.78 ^b ±0.03	3.50 ^c ±0.05
LM-30	76.76 ^a ±0.05	12.59 ^c ±0.09	3.59 ^c ±0.04	2.99 ^a ±0.03	4.07 ^b ±0.08
p Value	0.000	0.000	0.000	0.002	0.001

CP- Crude protein; TL - Total lipid; TA - Total ash; TC - Total carbohydrate

All values are expressed as mean±SE, n=3. Mean values in the same column with different superscript differ significantly (p<0.05).

molecules into micro particles that help in assimilation and utilisation in the body. The activity of digestive enzymes is known to be affected by nutrient composition (Kawai and Ikeda, 1972). Sabat *et al.* (1999) also indicated that modulation of digestive enzyme activity in response to diet is common but not universal among the vertebrates. In the present study, the protease and amylase activities did not vary significantly ($p>0.05$) among different treatments, while lipase activity showed a significant difference between the treatments (Table 6). A significantly higher ($p<0.05$) lipase activity was observed in the LM20 group compared to all other groups indicating better lipid digestion in this group. This can be correlated with the higher growth rate and lipid deposition observed in this group compared to all other groups.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are important aminotransferase enzymes that redistributes amino-nitrogen among amino acids. AST catalyses the inter-conversion of aspartate and α -ketoglutarate to oxaloacetate and glutamate. ALT transfers the amino group from alanine to α -ketoglutarate and convert to glutamic acid forming pyruvate. The pyruvate and oxaloacetate thus formed are responsible for the synthesis of non-essential amino acids like alanine, asparagine and glutamine, which in turn help in protein synthesis and enhance the growth of the animal. Apart from this, the amino acids are de-aminated to produce TCA

cycle intermediates and also help in energy production in the form of ATP (De Silva and Anderson, 1995). In the present study, AST activity of muscle was not significantly ($p>0.05$) different among the treatments, whereas liver AST activity showed a significant difference among the groups (Table 7). The lowest AST activity ($p<0.05$) was observed in the LM10 group and significantly higher activity was observed in LM20 and LM30 groups. Similarly, ALT activity in the liver was significantly higher in the LM20 group compared to other treatment groups. The higher hepatic AST and ALT activity in 20% BGLM fed group indicate that the increased utilisation of the glucose derived TCA intermediates for protein synthesis. This is also supported by the higher growth rate in this group.

Lactate dehydrogenase is the terminal enzyme of the glycolysis pathway. LDH converts lactate to pyruvate in the presence of coenzyme NADH which is converted to NAD^+ . Thus, lactate dehydrogenase helps in maintaining the glycolysis cycle by supplying NAD^+ . In the presence of enough oxygen, pyruvate enters the Krebs's cycle, but can be converted to lactate in the depletion of the tissue oxygen. In our study, significantly higher muscle LDH activity was found in the LM30 group, while other leaf meal fed groups showed similar activity with the control group (Table 8). This may be due to the metabolic stress generated due to the higher inclusion level of BGLM,

Table 6. Effect of leaf meal based diets on intestinal digestive enzymes activity in *L. rohita* fingerlings

Treatments	Protease	Amylase	Lipase
C	34.08 $\pm$ 5.79	0.29 $\pm$ 0.05	2.80 ^b $\pm$ 0.02
LM-10	30.27 $\pm$ 2.44	0.30 $\pm$ 0.02	3.98 ^b $\pm$ 0.52
LM-20	39.04 $\pm$ 3.37	0.27 $\pm$ 0.03	5.85 ^a $\pm$ 0.71
LM-30	45.82 $\pm$ 2.45	0.33 $\pm$ 0.01	3.15 ^b $\pm$ 0.47
p Value	0.085	0.620	0.011

Protease activity expressed as micromole tyrosine released ($\text{mg protein}^{-1} \text{ min}^{-1}$)

Amylase activity expressed in the terms of micromole maltose released ($\text{mg protein}^{-1} \text{ min}^{-1}$)

Lipase activity is expressed in units per mg protein.

All values are expressed as mean $\pm$ SE, n=3. Mean values in the same column with different superscripts differ significantly ($p<0.05$).

Table 7. Activity of protein metabolic enzymes in liver and muscle in the experimental groups fed with different experimental diets

Treatments	AST (M)	AST (L)	ALT (M)	ALT (L)
C	27.32 $\pm$ 0.35	25.07 ^b $\pm$ 0.96	43.58 $\pm$ 0.76	13.43 ^b $\pm$ 1.49
LM-10	23.45 $\pm$ 0.20	18.05 ^c $\pm$ 0.20	36.14 $\pm$ 2.24	12.42 ^b $\pm$ 0.79
LM-20	25.66 $\pm$ 2.64	32.23 ^a $\pm$ 0.69	40.73 $\pm$ 4.68	18.97 ^a $\pm$ 0.06
LM-30	31.09 $\pm$ 2.33	30.10 ^a $\pm$ 1.09	52.33 $\pm$ 1.28	8.22 ^c $\pm$ 0.97
p Value	0.139	0.001	0.053	0.000

L - Liver; M - Muscle.

AST : Specific activities expressed as nanomoles of oxaloacetate released $\text{min}^{-1} \text{ mg protein}^{-1}$.

ALT : Specific activities expressed as nanomoles of sodium pyruvate released $\text{min}^{-1} \text{ mg protein}^{-1}$.

All values are expressed as mean $\pm$ SE, n=3.

Mean values in the same column with different superscripts differ significantly ($p<0.05$).

Table 8. Activity of carbohydrate metabolic enzymes in liver and muscle in experimental groups fed with different experimental diets

Treatments	LDH (M)	LDH (L)	MDH (M)	MDH (L)
C	25.96 ^b ±0.24	12.39±2.14	1.97±0.21	2.28 ^a ±0.16
LM-10	21.97 ^b ±0.76	10.56±0.38	2.12±0.52	1.45 ^b ±0.24
LM-20	24.62 ^b ±1.72	13.98±1.36	2.09±0.16	1.59 ^b ±0.05
LM-30	33.05 ^a ±2.46	16.21±3.10	2.09±0.08	1.65 ^b ±0.06
p Value	0.028	0.360	0.983	0.019

L - Liver; M - Muscle.

LDH and MDH activity expressed as micromoles mg protein⁻¹ min⁻¹.

All values are expressed as mean±SE, n=3.

Mean values in the same column with different superscripts differ significantly (p<0.05).

which can be a reason for the lower growth rate in LM30 group. Malate dehydrogenase (MDH) is an enzyme of the citric acid cycle, which catalyses the conversion of malate into oxaloacetate and *vice versa*. Hepatic MDH activity was found to be significantly different (p>0.05) among the groups, with the highest value in the control group compared to other leaf meal fed groups (Table 8). MDH activity in muscle was found to be similar (p>0.05) among the different treatment groups. The higher activity of MDH in control group indicates greater activity of the TCA cycle due to increased energy demands (Wedemeyer *et al.*, 2000). Gbadamosi *et al.* (2016) reported that dietary *M. oleifera* extract in Nile tilapia decreased the MDH activity in the treatment groups compared to the control.

Results of the study clearly indicated that de-oiled rice bran can be completely replaced with *V. mungo* leaf meal (BGLM) in the diet of *L. rohita* fingerlings, in terms of growth performance and nutrient utilisation. However, maximum growth performance and nutrient utilisation can be obtained at 20% inclusion level in the diet of *L. rohita* fingerlings.

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