

Effect of *Mucuna bracteata* leaf meal diet on growth, digestion and metabolism in *Cyprinus carpio* (Linnaeus 1758)

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Abstract

The current study evaluated mucuna leaf meal (MLM) as an alternative for de-oiled rice bran (DORB) in the *Cyprinus carpio* fingerling diet. Following a complete randomised design, a 60-day feeding experiment was carried out, where four isonitrogenous (32% crude protein) and iso-caloric (356 kcal DE 100 g⁻¹) diets were prepared to constitute the following treatments; C (30% DORB, 0% MLM), T1 (20% DORB, 10% MLM), T2 (10% DORB, 20% MLM) and T3 (0% DORB, 30% MLM). Significant differences ($p < 0.05$) were observed among treatments in terms of weight gain percentage (WG), protein efficiency ratio (PER) and specific growth rate (SGR). The growth performance of *C. carpio* fingerlings in the 30% MLM group (T3) was comparable to that of the control group (C, 30% DORB). Notably, fish fed with T2 diets exhibited better growth compared to those in other treatments. Digestive enzyme activities remained largely unchanged, except for amylase activity, which showed a significant increase in the T2 group. Additionally, alanine aminotransferase (ALT) activity in both muscle and liver was significantly elevated ($p < 0.05$) in this group. These findings indicate that MLM can serve as an effective alternative to DORB in the diet without causing adverse effects, with the 20% MLM diet providing optimal growth and physiological benefits. However, malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) activities on hepatic and muscular tissues were significantly higher ($p < 0.05$) in 30% MLM groups. Based on this, it can be concluded that the MLM can fully replace DORB (30%) without generating any detrimental consequences in the diet of *C. carpio*.



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Introduction

Aquaculture significantly reduces poverty and enhances nutritional security, improving the socioeconomic conditions of impoverished people, especially in developing nations (FAO, 2018). According to the Food and Agriculture Organisation's (FAO, 2024) report, global fisheries and aquaculture production reached a record 223.2 million t in 2022, marking a 4.4% increase from 2020. Notably, aquaculture production of aquatic animals surpassed capture fisheries for the first time, contributing 94.4million t, accounting for 51% of the total aquatic animal production (FAO, 2024). This milestone underscores the growing significance of aquaculture in meeting global seafood demand. Aquafeeds need to incorporate alternative, locally available ingredients due to the increasing scarcity of conventional protein and

energy sources. In Asia, traditional carp feed primarily consists of oil cakes and de-oiled rice bran (DORB) (Meshram *et al.*, 2018). Farm-made carp diets often rely heavily on DORB, accounting for over 85% of their composition. This dependence raises concerns about its future availability, as DORB is also in demand for human consumption and terrestrial livestock feed (Maiti *et al.*, 2019). Given these challenges, identifying sustainable substitutes for DORB is essential to ensure the long-term viability of aquafeeds. Previous studies proposed that unused and wasted plant leaves in the form of dried meal may be a suitable option for replacing DORB in the fish diet (Meshram *et al.*, 2018; Ahmad *et al.*, 2019; Maiti *et al.*, 2019, Anand *et al.*, 2020; Sahoo *et al.*, 2020).

Plant leaves have certain limitations as aquafeed ingredients due to the presence of

anti-nutritional factors (ANFs), which can affect nutrient utilisation and overall fish health. Sweet potato, *Leucaena leucocephala*, *Morus esculenta*, *Medicago sativa*, *Moringa oleifera* and *Manihot esculenta* are the plant leaves that have lately been added to 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 maintain a steady supply across different seasons and regions, it is essential to evaluate a diverse range of leaf meals for their suitability in aquafeeds. This study explored the feasibility of using mucuna leaf meal (MLM) as an ingredient in the pelleted diets of common carp. Mucuna bean (*Mucuna pruriens* var. *utilis*), is a legume native to tropical regions, containing similar levels of nutrients such as protein, fat, minerals and vitamins (Ravindran, 1988; Siddhuraju *et al.*, 1996). According to Duke (1981), its value as a source of proteins for animal feeds is well known, especially in underdeveloped nations. The common carp *Cyprinus carpio*, 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 reducing the production cost, particularly the feed cost of common carp, is a challenge for the researchers; evaluating cost-effective feed alternatives, such as leaf meals, is crucial for optimising the diet of this commercially valuable species.

M. bracteata, an underutilised plant resource, has emerged as a promising alternative to DORB in aquafeeds. A review of the use of underutilised plants in aquaculture emphasised the role of *M. bracteata* in reducing the reliance on conventional feed resources. It also highlighted how incorporating such plants can contribute to biodiversity and sustainability within aquaculture systems by promoting the use of locally available and less exploited plant resources (Roshan *et al.*, 2022). Additionally, Studies on the nutritional properties of *M. pruriens*, a closely related species to *M. bracteata*, have revealed its high protein content, making it a valuable candidate for inclusion in animal feeds. These studies underlined the potential of *Mucuna* species as a protein-rich alternative while also recognising the importance of addressing its anti-nutritional factors for optimal use in aquafeeds (Roshan *et al.*, 2022).

Materials and methods

Leaf meal preparation

Leaves of *M. bracteata* DC. ex Kurz (Acc. No: 14825) were sourced from Calicut, Kerala. They were thoroughly washed with tap water and shade-dried for two days. Once fully dried, the leaves were finely ground using a mixer grinder and stored in an airtight container at the Fish Nutrition Laboratory, ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Mumbai, India.

Proximate analysis of diets

The proximate composition of the experimental diets was analysed at the Fish Nutrition Laboratory, ICAR-CIFE, Mumbai, following the standard protocols outlined by AOAC (1995). Moisture content was determined by recording the weight of shade-dried leaves before and after drying at 105°C overnight. Crude protein and

lipid levels were estimated using the Micro-Kjeldahl method and Soxhlet extraction, respectively. The ash content was assessed by incinerating the samples in a muffle furnace set at 450°C, followed by weighing the remaining ash residue. Crude fiber content (CF%) was analysed through acid and alkali digestion procedures. The nitrogen-free extract (NFE%) was calculated using the formula: $NFE\% = 100 - [Crude\ protein\ (\%) + Ether\ extract\ \% + Crude\ fibre\ (\%) + Total\ ash\ (\%)]$.

Experimental diets

To formulate four nutritionally balanced diets with equal protein (32% crude protein) and energy content (356 Kcal digestible energy per 100 g), the required ingredients were precisely weighed (Table 1). These ingredients were thoroughly mixed to ensure uniformity. The resulting mixture was steam-cooked at 121°C for 25 min to gelatinise starch, thereby enhancing pellet stability. Once cooled, essential additives, including oil, butylated hydroxytoluene, a vitamin-mineral premix, and choline chloride, were incorporated. The prepared mixture was then processed into pellets using an automatic pelletiser (SB Panchal and Company, Mumbai). The pellets underwent an initial air-drying phase at room temperature under a fan for 24 h, followed by controlled drying in an oven at 40°C for one hour to achieve the desired moisture content. The final feed was cooled and stored in airtight containers at 4°C until use.

Procurement and acclimatisation of experimental animals

Common carp fingerlings were transported to the wet laboratory facility at ICAR-CIFE, Mumbai and housed in two FRP circular tanks with continuous aeration. To facilitate acclimatisation, the fish were provided with a standard diet containing 32% crude protein and 6% lipid for 21 days before the commencement of the feeding trial.

Experimental design

The feeding trial was conducted in the wet laboratory of ICAR-CIFE using tanks with a water-holding capacity of 175 l. Fingerlings, averaging 6.06-6 cm in length, were distributed among the tanks and provided with their respective experimental diets twice daily at 09:00 and 17:00 hrs. To maintain water quality, uneaten feed and waste matter were carefully removed by siphoning, followed by replenishment with an equivalent volume of freshwater.

Digestibility studies

Chromic oxide (Cr_2O_3) was incorporated as an external marker at a concentration of 5 g kg⁻¹ in the diet to facilitate digestibility assessment using an indirect method during the final 30 days of the feeding trial (Alexander *et al.*, 2011). The apparent digestibility coefficients (ADCs) for nutrients were calculated using the following formulae and presented as a direct ratio:

$$ADC\ of\ dry\ matter = 100 \left(1 - \frac{\%Chromic\ oxide\ in\ diet}{\%Chromic\ oxide\ in\ faeces} \right)$$

$$ADC\ of\ nutrients = 100 \left(1 - \frac{\%Chromic\ oxide\ in\ diet \times \%nutrient\ in\ faeces}{\%Chromic\ oxide\ in\ faeces \times \%nutrient\ in\ the\ diet} \right)$$

Growth performance and nutrient utilisation

After completing the 60-day feeding trial, fish from each tank were carefully collected and anaesthetised using clove oil at a concentration of 50 mg l⁻¹ (Keene *et al.*, 1998). Individual weights were recorded to assess growth performance. Key parameters, including weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR), were determined using standard formulae:

$$\text{WG} = \frac{\text{Final live weight (g)} - \text{Initial live weight (g)}}{\text{Initial live weight (g)}} \times 100$$

$$\text{SGR} = \frac{(\ln \text{ of final wet weight} - \ln \text{ of initial wet weight})}{\text{Experimental duration in (days)}} \times 100$$

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

$$\text{PER} = \frac{\text{Final wet weight gain (g)}}{\text{Protein intake (g) on dry matter basis}}$$

$$\text{Survival (\%)} = \frac{\text{Stocking density at start of experiment} - \text{No of live fish at end of experiment}}{\text{Stocking density at start of experiment}} \times 100$$

Estimation of digestive and metabolic enzymes

On termination of the feeding trial, two fish from each replicate were sedated using clove oil (50 mg l⁻¹), after which liver and muscle tissues were carefully excised. These tissues were homogenised in chilled 0.25M sucrose solution using a mechanical homogeniser, ensuring that the process was carried out on ice to maintain enzyme stability. The homogenates were then centrifuged at 5000 rpm for 10 min and the resulting supernatants were collected in sterile vials and preserved at -20°C for further analysis. Protease activity was assessed through casein digestion, with results expressed in millimoles of tyrosine released per gram of protein per minute (Drapeau, 1976). Amylase activity was assessed by Rick and Stegbauer's (1974) procedure, by evaluating starch hydrolysis, expressed as the amount of maltose released per minute per gram of protein. Lipase activity was determined through Cherry and Crandall's (1932) titrimetric approach, with enzyme activity represented in units per hour per milligram of protein. Lactate dehydrogenase (LDH) activity in muscle and liver tissues was estimated following the method outlined by Wroblewski and LaDue (1955), with the results expressed in micromoles of NAD⁺ released per milligram of protein per minute. Similarly, malate dehydrogenase (MDH) activity was determined based on the oxidation of NADH, measured in micromoles per milligram of protein per minute, differing from LDH in terms of the substrate used.

Statistics analysis

The experiment followed a completely randomised design, and the collected data were analysed to determine the mean and standard error. A one-way analysis of variance (ANOVA) was conducted

using SPSS software (version 16) with a 95% confidence interval to assess statistical differences. Duncan's multiple range test was applied to compare mean values and identify significant variations among treatments.

Results and discussion

Proximate analysis of diets

The formulation and proximate composition of experimental diets are given in Table 1 (Roshan *et al.*, 2022). 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 fibre (7.14-7.74%), ash content (8.45-8.87%), and total carbohydrate (43.99-46.34%) showed no significant ($p > 0.05$) alterations (NRC, 2011).

Digestibility studies

A major challenge that limits the use of alternative feed sources of plant origin is fish feed acceptability, which is typically associated with diet's palatability (Rumsey, 1993). Incorporating MLM at a 20% inclusion level in the diet of *C. carpio* significantly enhanced the apparent dry matter digestibility coefficient (ADMDC) compared to the control group ($p < 0.05$) (Table 2). Among the dietary groups, the apparent crude protein digestibility coefficient (ACPDC) and apparent lipid digestibility coefficient (ALDC) did not differ significantly ($p > 0.05$), aligning with previous findings that alternative plant-based meals can maintain nutrient digestibility in fish (Gatlin *et al.*, 2007; Kumar *et al.*, 2018).

Growth and nutrient utilisation of *Cyprinus carpio* fingerlings

In this study, the inclusion of MLM at a 20% dietary level significantly improved weight gain and specific growth rate compared to the control group. Furthermore, incorporating MLM at levels up to 30% in the diet of common carp effectively replaced DORB without negatively impacting weight gain percentage, specific growth rate, or feed conversion ratio (Table 3). Previously, the experimental diets using mucuna seed meal demonstrated high acceptability and no essential amino acid (EAA) deficits (Siddhuraju *et al.*, 1996). Similarly, raw sweet potato leaf meal (Meshram *et al.*, 2018) and *Hygrophila spinosa* leaf meal (Maiti *et al.*, 2019) have been shown to be capable of replacing DORB completely in the diet of *Labeo rohita*, when incorporated at a level of 30%. Further, Amisah *et al.* (2009) found that inclusion of *L. leucocephala* leaf meal at 30% had no impact on the growth of *Clarias gariepinus*.

Estimation of digestive and metabolic enzymes

The ability of fish to hydrolyse complex nutritional compounds in feed into absorbable molecules is attributed to digestive enzyme activity (Sahoo *et al.*, 2020). The activity of these digestive enzymes can be influenced by changes in feed intake (Steffens, 1987), nutrient make-up of the feed (Perez-Jimenez *et al.*, 2009; Krogdahl *et al.*, 2010) and water temperature (Lazzari *et al.*, 2010). The digestibility of dietary constituents like protein, fat and carbohydrates in fish positively correlates with the activity

Table 1. Formulation of experimental diets and proximate composition

Ingredients 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 and 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 (on 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 fibre (%)	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 ¹⁰ (%)	368.54	367.54	365.19	358.44
P:E ¹¹ (%)	87.04	87.03	87.87	89.11

*The mean results of triplicates were used to calculate the proximate composition

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. ²DSBM, De-oiled soybean meal; ³GNOC, Groundnut oil cake; ⁴DORB, De-oiled rice bran; ⁵MLM, *Mucuna bracteata* leaf meal. ⁶Composition 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 K, 1,000 mg; Vitamin B₁, 1,000 mg; Vitamin B₁₂, 6 mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 1 g; Choline chloride, 150 g; Mn, 27,000 mg; I, 325 mg; 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. ⁷CMC, Carboxymethyl cellulose; ⁸BHT = Butylated hydroxytoluene

Table 2. Apparent digestibility coefficient of nutrients

Treatments	ADMDC ¹	ACPDC ²	ALDC ³
Control	0.52 ^a ±0.01	0.80±0.03	0.96±0.07
MLM10	0.56 ^{ab} ±0.01	0.82±0.02	0.96±0.06
MLM20	0.58 ^c ±0.01	0.83±0.01	0.96±0.04
MLM30	0.55 ^{ab} ±0.02	0.79±0.01	0.96±0.08

Data represent mean±SE (n=6). Mean values in the same column with different superscripts differ significantly (p<0.05). ¹ADMDC- Apparent dry matter digestibility coefficient, ²ACPDC- Apparent crude protein digestibility coefficient, ³ALDC- Apparent lipid digestibility coefficient

Table 3. Growth parameters of *C. carpio* fingerlings reared for a period of 60 days, under various experimental diets*

Treatments ¹	IBW ² (g)	FBW ³ (g)	WG ⁴	SGR ⁵	PER ⁶
Control	6.06±0.01	12.05±0.06	79.82±1.33	0.98±0.01	1.38±0.05
MLM10	6.07±0.02	12.69±0.19	101.41±0.68	1.17±0.02	1.51±0.02
MLM20	6.01±0.05	12.88±0.12	133.27±0.69	1.43±0.01	1.73±0.01
MLM30	6.04±0.04	12.10±0.11	81.53±0.71	0.99±0.02	1.40±0.03

* The data are presented as Mean±SE (n=6). Mean values within the same column that have different superscripts indicate significant differences (p<0.05). The treatment groups include: Control (30% DORB and 0% MLM), MLM10 (10% MLM replacing 33.3% DORB and 23.4% GNOC), MLM20 (20% MLM replacing 66.6% DORB and 46.8% GNOC), and MLM30 (30% MLM replacing 100% DORB and 70.2% GNOC). 2IBW: Initial body weight; 3FBW: Final body weight; 4WG: Percent weight gain; 5SGR: Specific growth rate; 6PER: Protein efficiency ratio

of corresponding digestive enzymes like protease, lipase and amylase (Cho and Slinger, 1979; De *et al.*, 2015). In this study, the amylase activity in the MLM10, MLM20 and MLM30 groups was notably higher (p<0.05) than that observed in the control group. This enhanced enzymatic activity that the dietary carbohydrates in MLM are more bioavailable, leading to improved carbohydrate metabolism and energy production. Protease and lipase enzyme activity did not show significant differences (p>0.05) among the dietary groups in this study (Table 4). This may be due to the experimental diets being isonitrogenous and isolipidic (Yengkokpam *et al.*, 2013; Fawole *et al.*, 2016; Garg *et al.*, 2019).

Table 4. Activities of digestive enzymes of *carpio* fingerlings fed with different experimental diets for 60 days

Treatments	Protease ¹	Amylase ²	Lipase ³
Control	0.21±0.01	9.652±0.01	0.20±0.01
MLM10	0.22±0.01	10.52±0.08	0.22±0.03
MLM20	0.24±0.02	11.54±0.05	0.20±0.02
MLM30	0.21±0.01	10.82±0.03	0.21±0.03
p-value	0.133	0.000	0.652

All values are presented as mean±SE (n=6). Mean values within the same column bearing different superscripts indicate significant differences (p<0.05).

¹Protease activity (millimoles of tyrosine released mg protein⁻¹ min⁻¹)

²Amylase activity (micromoles of maltose released mg protein⁻¹ min⁻¹)

³Lipase activity (units mg protein⁻¹ h⁻¹)

A significant variation ($p < 0.05$) was observed in muscle and hepatic LDH and MDH activity levels among the dietary groups (Table 5). The inclusion of MLM at 30% in the diet led to a significant reduction ($p < 0.05$) in both muscle LDH and MDH activities compared to the control group. However, hepatic MDH activity showed a significant increase in the MLM-fed groups. Elevated LDH activity in the liver and muscle of the control group suggests that DORB may have induced anaerobic stress in fish, potentially leading to increased lactate production for energy metabolism. Previous studies have reported an increase in LDH activity under stressful conditions (Vijayaraghavan and Rao, 1986; Lakshmaiah, 2016).

Table 5. Activities of carbohydrate metabolic enzymes in *C. carpio* fingerlings fed with different experimental diets for the period of 60 days

Treatments	LDH ¹		MDH ²	
	Liver	Muscle	Liver	Muscle
Control	4.53 ^a ±0.01	12.07 ^c ±0.02	0.54 ^a ±0.01	0.12 ^a ±0.01
MLM10	4.34 ^b ±0.02	10.44 ^b ±0.15	0.77 ^{ab} ±0.14	0.08 ^b ±0.01
MLM10	3.48 ^a ±0.03	10.41 ^b ±0.12	0.80 ^b ±0.06	0.02 ^a ±0.01
MLM10	4.02 ^b ±0.02	9.66 ^a ±0.19	0.91 ^b ±0.01	0.05 ^{ab} ±0.03
p-value	0.001	<0.000	<0.039	<0.014

Data are presented as mean±SE (n=6). Mean values within the same column bearing different superscripts indicate significant differences ($p < 0.05$).

¹LDH, activity (micromoles of NAD released mg protein⁻¹ min⁻¹)

²MDH, activity (units mg protein⁻¹ min⁻¹)

Incorporating moringa leaf meal as a replacement for DORB in aquafeeds has significant economic advantages in aquaculture. DORB, a widely used feed ingredient, is subjected to price volatility due to its demand in multiple industries, including livestock feed and biofuel production. This fluctuation can strain the cost-efficiency of aquafeeds. Moringa leaf meal, on the other hand, is a sustainable alternative, particularly in regions where moringa is extensively cultivated. Its use could reduce reliance on DORB and stabilise feed costs, offering economic relief to aquaculture operations (Hussein, 2017). The enhanced apparent dry matter digestibility coefficient (ADMD) of MLM, along with its ability to sustain or improve key growth parameters such as specific growth rate (SGR) and feed conversion ratio (FCR) at inclusion levels of up to 30%, highlights its potential as a valuable ingredient in fish feed formulations. By promoting better feed utilisation, MLM may offset initial costs associated with its production or procurement. The absence of significant differences in protein and lipid digestibility coefficients (ACPD and ALDC) suggested that MLM-based feeds can sustain fish health and performance without adding expensive feed additives to counteract the anti-nutritional effects.

Mucuna leaf meal (MLM) presents a viable alternative to de-oiled rice bran (DORB) in the diet of *C. carpio* fingerlings, with no adverse effects on growth performance observed when replacing DORB entirely. The nutritional profile of MLM supports enhanced growth and digestive function. However, further studies are necessary to evaluate the long-term effects of MLM inclusion on growth rates and potential risk factors, particularly in grow-out trials.

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