



Effect of replacement of de-oiled rice bran with sweet potato leaf meal on growth performance, digestive enzyme activity and body composition of *Labeo rohita* (Hamilton, 1822)

ZISHAN AHMAD, ASHUTOSH DHARMENDRA DEO, SARVENDRA KUMAR*,
AMIT RANJAN, Md. AKLAKUR AND NAROTTAM PRASAD SAHU

ICAR-Central Institute of Fisheries Education, Versova, Mumbai - 400 061, Maharashtra, India

*College of Fisheries Science Gumla, Birsa Agricultural University, Ranchi - 834 006, Jharkhand, India

e-mail: ashutoshddeo@gmail.com; ashutosh@cife.edu.in

ABSTRACT

A 60-days feeding experiment was conducted to evaluate the utilisation of sweet potato leaf meal (SPLM) for replacement of de-oiled rice bran (DORB) in the diets of rohu *Labeo rohita* (Hamilton, 1822) fingerlings. SPLM has good protein content (22.12%) and digestible energy content (11.81 MJ kg⁻¹). Five iso-nitrogenous (30%) and iso-caloric diets (13.5 MJ kg⁻¹) were prepared by replacing DORB with sweet potato leaf meal (SPLM) at 0% (C), 25% (T1), 50% (T2), 75% (T3) and 100% (T4). Each dietary treatment was tested in triplicates with 12 fingerlings per tank, following completely randomised design. The growth performance and feed utilisation parameters viz., weight gain % (WG), specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR) were not significantly different (p>0.05) among treatments. The digestive enzyme activity remained unaffected except amylase activity, which increased significantly in 50% SPLM replacement group (T2). Chymotrypsin decreased significantly (p<0.05) in the treatment groups. Metabolic enzymes viz., ATPase activity of liver increased significantly (p<0.05) whereas ALP (alkaline phosphatase) activity in the intestine had irregular pattern. The present study indicates that DORB can be completely replaced by SPLM without affecting growth performance and digestive enzyme activity in the diet of *L. rohita*.

Keywords: Body composition, De-oiled rice bran, Growth performance, *Labeo rohita*, Sweet potato leaf meal

Introduction

Aquaculture is a rapidly growing food-producing sector. For sustainable fish production, use of formulated feed is of utmost importance. Aquaculture in India is slowly shifting from non-feed based or extensive culture systems to feed based semi-intensive culture systems. Artificial feeding is one of the most important factor required for faster growth and higher yield for fish culture (Jose *et al.*, 2006; Maity and Patra, 2008). The feed ingredients have to be sourced mainly from plants, agriculture products and animal protein. Plant-based alternative ingredients are widely available and many are already being used for aqua feed. The long-term viability of aquaculture will only be realised if use of alternative and non-conventional ingredients are promoted to use in place of conventional ingredients.

De-oiled rice bran (DORB) is the most commonly utilised agricultural byproducts used in feed formulation (Kumar *et al.*, 2017; 2018a,b; 2019; Ranjan *et al.*, 2018a,b; 2019). As a source of carbohydrate, it provides energy in the diet and also offers a substantial part of the essential amino acids (Tsvetanov and Duneva, 1990; Ranjan

et al., 2017; 2018a). Even in farm-made feed, major part is contributed by the rice bran (50-80%) along with other agricultural byproducts. The importance of dietary and functional properties of rice bran has led to its multiple uses including human consumption (Sharif, 2009) and so the demand for rice bran has grown exponentially. Further, yield losses in rice production due to climate change related issues became another determinant of price of rice bran. The cumulative effects of these multiple factors have forced the price of rice bran to surge up to ₹14-19 kg⁻¹ in Indian markets creating huge stress on aquafeed production. Hence, an alternative source of rice bran has been investigated here. Many reports on use of various sources of leaf meal proteins in diet of herbivorous and carnivorous fishes viz., cassava leaf meal (Ng and Wee, 1989), alfalfa (Yousif *et al.*, 1994), *Carica papaya* (Reyes and Fermin, 2003) and *Leucaena leucocephala* (Bairagi *et al.*, 2004). These studies projected leaf meal as a good source to replace protein and energy sources in fish feed. There are many inexpensive, unutilised leafy plants whose nutritive and anti-nutritive potentials are yet to be sufficiently studied and exploited, among which sweet potato leaves deserve a special attention

(Antia *et al.*, 2006). Sweet potato is a widely grown crop in tropical countries but originated from Central America. The crude protein and crude fibre contents of the foliage of sweet potato differ largely with variety, soil and water condition and parts of the plant. According to Preston (2006), the crude protein content of leaves varied from 26.5 to 32.5% on dry matter basis. Sweet potato leaves (SPL) are used as vegetables, especially in China and South-east Asia. Moreover, being used for human consumption, the SPL serves as fodder for cattle, sheep, goats, pigs and other domestic animals (Antia *et al.*, 2006). There are very few reports available on the use of sweet potato leaves in fish feed. The foliage production of sweet potato can be from 4.3 to 6.0 t DM ha⁻¹crop⁻¹ (Ruiz *et al.*, 1981). It can be planted twice in a year because it has a short generation interval hence it can be harvested many times throughout the year (Hong *et al.*, 2003). The price calculated at farm site is around ₹10 per kg and dry weight recovery was around 20% depending on the maturity stages of leaf. The shrubby parts of sweet potato, such as leaves, stalks and stems have a high nutritive value (Ishida *et al.*, 2000). In particular, leaves contain a large amount of protein with a high amino acid score. Contents of minerals and vitamins such as A, B₂, C and E are high in leaves in comparison to other vegetables. Presence of anti-nutritional factor is the major limiting factor of sweet potato leaf meal utilisation as feed ingredients. SPLM has been reported to contain various anti-nutritional factors like invertase, protease inhibitors, cyanide, tannins, oxalate and phytate (Tacon, 1993; Antia *et al.*, 2006). Sweet potato leaves are deficient in some essential amino acids like lysine, necessitating the inclusion of adequate lysine in diets (Fuller and Chamberlain, 1982). Since there is limited information available on the utilisation of sweet potato leaf meal as an energy source in the diet of rohu *Labeo rohita* (Hamilton, 1822), the present study was conducted to evaluate the potential of sweet potato leaf meal as a replacer of DORB in the diet.

Materials and methods

Preparation of sweet potato leaf meal (SPLM)

Sweet potato leaves were collected from the Doab region of Ganga basin near Patna from a local farm. The leaves were washed, dried in shade and brought to the lab and again dried in the oven at 40°C for a night and dried leaf was ground and screened using 50 mm mesh.

Experimental set up

Rohu fingerlings were procured from Nisarga fish farm, Boiser, Thane, Mumbai, Maharashtra. The fishes were transported to the wet laboratory of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Mumbai in oxygen filled polyethylene bags. Two hundred fifty

fishes were carefully transferred to a circular tank (500 l) and were left undisturbed overnight. In order to reduce handling stress, the fishes were given a vitamin C (1 mg l⁻¹) treatment and on the subsequent day salt treatment (20 g l⁻¹) was given to eliminate chances of parasitic infection. The fingerlings were acclimatised under aerated conditions for a period of 30 days. During acclimation, fish were fed with a basal diet containing 30% crude protein. One hundred eighty fingerlings with an average size of 4.3±0.2 g were randomly distributed in 15 rectangular plastic tanks following completely randomised design (CRD). The experiment was conducted for a period of 60 days. Five iso-caloric (13.5 MJ kg⁻¹) and iso-nitrogenous (30% crude protein) experimental diets were prepared (Table 1). Fishes were fed twice daily at 2% body weight at 10:00 hrs in the morning and in the evening at 16:00 hrs. Sufficient aeration was provided in all tanks. Siphoning was done daily to remove the excreta and residual feed. The same volume of water was replenished after siphoning.

Table 1. Proximate composition of sweet potato leaf meal (SPLM) and de-oiled rice bran (DORB)

Parameters	SPLM	DORB
Dry matter	95.57±5.00	91.93±0.60
Crude protein	22.12±3.00	15.03±0.50
Crude fat	3.59±1.50	0.57±0.18
Crude fiber	15.43±2.00	14.47±0.23
Nitrogen free extract	38.40±3.30	55.89±0.36
Ash content	16.03±6.50	6.07±0.61

Values are Mean±SE (n=3)

The ingredient composition of different experimental diet is presented in Table 2. Practical ingredients such as groundnut oil cake, de-oiled rice bran, wheat flour, carboxymethyl cellulose (CMC), defatted soyabean meal, butylated hydroxytoluene (BHT), cod liver oil, vitamin and mineral mixture, were used for feed preparation. Six iso-caloric and iso-nitrogenous diets were prepared with 30% protein. All the ingredients were weighed according to the formulation, cooked in pressure cooker, cooled, vitamins and mineral mixture were added, pressed through a pelletizer and dried. After drying, the pellets were packed in polythene bags and were sealed airtight and were labelled according to the treatments.

Water quality parameters

Water quality parameters *viz.*, temperature, dissolved oxygen, ammonia, total hardness, pH, ammonia, nitrite-N and nitrate-N were determined as per the standard methods of APHA (1998).

Table 2. Ingredients composition of experimental diets

Ingredients	C	T1	T2	T3	T4
GNOC ^a	30	28	26	26	26
SPLM ^b	0	7.5	15	22.5	30
DORB ^c	30	22.5	15	7.5	0
DSBM ^d	20	20	20	19	19
Wheat flour ^e	9.35	11.35	13.35	14.3	14.8
Fish meal ^e	5	5	5	5	4.5
Fish oil ^e	3	3	3	3	3
Vit-min mix ^f	2	2	2	2	2
Vit C ^g	1.0	1.0	1.0	1.0	1.0
BHT ^g	0.05	0.05	0.05	0.05	0.05
CMC ^g	0.5	0.5	0.5	0.5	0.5

T: Treatment; C: Control; CMC: Carboxymethyl cellulose; BHT: Butylated hydroxyl toluene; ^aGNOC: Groundnut oil cake; ^bSPLM: sweet potato leaf meal; ^cDORB: De-oiled rice bran; ^dDSBM: Defatted soybean meal; ^ePurchased from Vaighai agro products, India; ^fProcured from local retail shop.

^f Composition of vitamin mineral mix (PREEMIX PLUS) (quantity per kg): Vitamin A - 55,00,000 IU; Vitamin D₃ - 11,00,000 IU; Vitamin B₂ - 2,000 mg; Vitamin E - 50 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^g Purified ingredients procured from HiMedia Ltd., India

Proximate analysis of leaf, diet and whole fish

Proximate analysis of the DORB, SPLM and experimental diets, was done by following standard methods (AOAC, 1995). Similarly, body composition of experimental fishes was estimated following AOAC method (1995). Moisture content of the experimental diets was determined by taking a known weight of the sample in a petridish and drying it in a hot air oven at 100-105°C till a constant weight was achieved. Crude protein content was determined by micro-Kjeldahl method (Kelplus, PELICAN, India). Fat content of experimental diets samples were estimated by soxhlet apparatus using diethyl ether (boiling point 65±5°C) as the solvent. Ash content of the experimental diets was estimated by taking a known weight of dried samples in a silica crucible and placing it in a muffle furnace at 600°C for 5-6 h. Fibre estimation was done in Fibre Tech (Tulin Equipment, India) apparatus and further ashing was done using a muffle furnace at 550°C for 5 h. Nitrogen free extract of the experimental diets was calculated by subtracting the percentage of other nutrients from 100:

$$\text{NFE (\%)} = 100 - [\text{Crude protein (\%)} + \text{Ether extract (\%)} + \text{Ash (\%)} + \text{Fibre (\%)}]$$

Growth performance and nutrient utilisation

The growth assessment of experimental fish were done fortnightly by estimation of growth parameters and nutrient utilisation parameters using following formulae:

Weight gain %	= [Final weight - Initial weight / Initial weight] × 100
Specific growth rate (SGR)	= [(Log _e Final weight - Log _e Initial weight) / Number of days] × 100
Feed conversion ratio (FCR)	= [Feed consumed (g, on dry weight basis) / Body gain (g, on wet weight basis)]
Feed efficiency ratio (FER)	= [Body gain (g on wet weight basis) / Feed consumed (g on dry weight basis)]
Protein efficiency ratio (PER)	= [Net weight gain (g on wet weight basis) / Protein fed (g on dry weight basis)]
Hepatosomatic index (HSI)	= [Wet weight of liver (g) / Wet weight to fish (g)] × 100
Intestinal somatic index (ISI)	= [Weight of intestine (g) / Weight of fish] × 100

Enzyme assay

Sample preparation

On termination of the experiment, three fishes from each tank were randomly collected and anaesthetised with clove oil (50 µl l⁻¹). Fishes were then dissected and the tissues *viz.*, liver, intestine and muscle, were immediately excised. A 5% tissue homogenate was prepared in chilled 0.25 M sucrose solution using a teflon coated mechanical homogenizer (REMI, Mumbai, India). The whole procedure was carried out in ice-cold condition. Homogenised samples were centrifuged at 6000 rpm for 10 min at 4°C. The supernatant was collected in glass vials and stored in a deep freezer (-20°C) for enzyme assay. Total protein of each tissue sample for enzyme assays was estimated by the Bradford method (Bradford, 1976).

Digestive enzymes assay

Protease activity was determined by the casein digestion method (Drapeau, 1976). Trypsin and chymotrypsin activity were estimated as per the methods described by Kunitz *et al.* (1947). Amylase activity was estimated as the reducing sugars produced due to the action of glucoamylase following di-nitro-salicylic-acid (DNS) method (Rick and Stegbauer, 1974). Specific amylase activity was expressed as the mole of maltose released from starch per min at 37°C temperature. The lipase activity was determined following the procedure of Cherry and Crandall (1932). The reaction mixture was prepared with distilled water, tissue homogenate, phosphate buffer (pH 7) and olive oil emulsion. The whole mixture was shaken well and incubated at 37°C for 24 h. After adding 95% alcohol and 2 drops of phenolphthalein indicator, titrated against 0.05 N NaOH until it exhibited

a permanent pink colour. The milli-equivalent of alkali consumed was taken as a measure of the activity of the enzyme unit mg^{-1} protein.

Alkaline phosphatase (ALP) assay

The alkaline phosphatase (ALP) activity was determined by the method of Garen and Levinthal (1960). The assay mixture comprised 0.2 ml bicarbonate buffer (0.2 M), 0.1 ml of 0.1 M MgCl_2 , 0.1 ml tissue homogenate, 0.5 ml of distilled water and 0.1 ml freshly prepared 0.1 M para-nitrophenyl phosphate. The reaction mixture was incubated in a water bath at 37°C for 15 min and the reaction was stopped by adding 1.0 ml of 0.1 N NaOH and OD was measured at 410 nm.

Adenosine triphosphate diphosphohydrolase (ATPase) assay

ATPase (Adenosine triphosphate diphosphohydrolase, E.C. 3.6.1.3) was assayed in a reaction mixture of 0.1 M Tris-HCl buffer (pH 7.8), 100 mM NaCl, 20 mM KCl, 3 mM MgCl_2 and 5 mM ATP. The mixture was incubated for 15 min and then the reaction was terminated by means of 10% trichloroacetic acid (Post and Sen, 1967). Phosphate liberated was estimated at 660 nm (Fiske and Subbarow, 1925).

Statistical analysis

The data was subjected to one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows. Duncan's multiple range test was performed for *post hoc* comparison of means between different experimental groups. All data presented are means \pm standard error and

statistical significance for all statistical tests was set at $p < 0.05$.

Results and discussion

The water quality parameters such as temperature ($27.3\text{--}29.5^\circ\text{C}$), dissolved oxygen ($6\text{--}6.9 \text{ mg l}^{-1}$), ammonia ($0.13\text{--}0.20 \text{ mg l}^{-1}$), total hardness ($229\text{--}246 \text{ mg l}^{-1}$), pH ($7.2\text{--}8.5$), nitrite-N ($0.001\text{--}0.002 \text{ mg l}^{-1}$), nitrate-N ($0.03\text{--}0.04 \text{ mg l}^{-1}$) recorded during the experimental period were found to be within the optimum range for fish.

The proximate composition of SPLM and DORB are presented in Table 1. As compared to DORB, SPLM contains more crude protein and crude fat. However, SPLM contains more crude fibre and total ash content. The proximate composition of experimental diets is presented in Table 3. The proximate composition of whole body of *L. rohita* is presented in Table 4. The body composition of *L. rohita viz.*, moisture content, dry matter, crude protein, lipid, carbohydrate and ash content did not vary significantly ($p > 0.05$).

Feed cost represents the highest operating cost in aquaculture, ranging from 30 to 60% of the operating cost based on the culture system (Riaz, 1997). The cost of feed can be reduced by using low cost ingredients. The evaluation of ingredient is based on nutritional composition and its effect on growth performance of fish. The proximate composition (%) of sweet potato (*Ipomoea batatas*) leaves showed that ash, crude fat, crude protein, crude fibre and nitrogen-free extract content were lower than the values reported by

Table 3. Proximate composition of experimental diets

Ingredients	C	T1	T2	T3	T4
Dry matter	90.96 \pm 0.76	91.12 \pm 0.19	89.90 \pm 0.62	90.89 \pm 0.15	91.92 \pm 0.96
Crude protein	31.05 \pm 0.06	30.57 \pm 0.02	30.95 \pm 0.07	30.63 \pm 0.16	30.63 \pm 0.87
Crude lipid	5.89 \pm 0.83	5.95 \pm 0.96	5.00 \pm 0.37	5.84 \pm 0.58	5.41 \pm 0.66
Ash	9.26 \pm 0.64	8.73 \pm 0.44	9.35 \pm 0.67	8.49 \pm 0.55	10.66 \pm 0.36
Dietary fiber	8.95 \pm 0.34	9.18 \pm 0.86	8.34 \pm 0.02	9.70 \pm 0.08	10.46 \pm 0.40
NFE	33.78 \pm 0.36	36.66 \pm 0.50	34.24 \pm 0.22	36.21 \pm 0.45	30.72 \pm 0.04
Energy(MJ kg^{-1})	13.58 \pm 0.83	13.64 \pm 0.12	13.25 \pm 0.44	13.69 \pm 0.01	13.12 \pm 0.67

C: Control; T: Treatments; NFE: Nitrogen free extract, Calculated energy* (MJ kg^{-1}) = $[16.74 \text{ CP (g kg}^{-1}) + 37.7 \text{ EE (g kg}^{-1}) + 16.74 \text{ TC (g kg}^{-1})] / 1000$

Table 4. Proximate composition of whole body of *L. rohita* fingerlings of different experimental groups (% wet weight basis)

Treatment	Moisture	Dry matter	Crude protein	Lipid	Carbohydrate	Ash
Control	76.93 \pm 0.42	23.06 \pm 0.73	14.84 \pm 0.49	2.34 \pm 0.29	2.21 \pm 0.26	3.65 \pm 0.66
T1	77.09 \pm 0.46	22.90 \pm 0.80	14.95 \pm 0.16	2.70 \pm 0.24	3.24 \pm 0.43	2.63 \pm 0.30
T2	77.33 \pm 0.42	22.67 \pm 0.74	14.97 \pm 0.73	2.90 \pm 0.09	2.29 \pm 0.97	3.29 \pm 0.56
T3	76.94 \pm 0.74	23.06 \pm 1.28	13.91 \pm 0.65	2.69 \pm 0.08	3.13 \pm 0.39	3.31 \pm 0.36
T4	76.92 \pm 0.49	23.67 \pm 0.86	15.34 \pm 0.35	2.78 \pm 0.34	1.84 \pm 0.22	3.32 \pm 0.43
p value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

All values are expressed as Mean \pm SE (n=3)

Hong *et al.* (2003), Akindahunsi and Salawu (2005) and Etuk *et al.* (2009). However, protein content recorded during the present study was similar to that reported by Oduro *et al.* (2008), but less than that observed by Antia *et al.* (2006). It may be due to the difference in variety as reported by Ishida *et al.* (2000), who found significant differences in protein content among the cultivars. The high ash content (16.03 ± 0.65) in the SPLM is a reflection of the high mineral contents. The results, therefore suggest a high deposit of mineral elements in the leaves.

There was no significant effect of dietary DORB replacement by SPLM on the tissue protein level and in the lipid level of the fish. This is supported by the findings of Muin *et al.* (2013) who reported that the replacement of rice bran with *Pleurotus florida* stalks has no significant impact on body composition of *Clarias gariepinus* fingerlings. In the present study these values were within the normal range for *L. rohita*, which was in agreement with Adewolu *et al.* (2008) who reported that SPLM did not cause any significant change in the body composition of *Tilapia zilli* fingerlings.

The nutritional quality of sweet potato leaf meal in rohu was determined in terms of body weight gain (WG %), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). The growth and nutrient utilisation parameters *viz.*, WG (%), SGR, FCR and PER are presented in Table 4. The weight gain percentage, SGR, FCR and PER did not vary significantly ($p > 0.05$) among the various treatment groups (Table 4). However, these parameters were not significantly different ($p > 0.05$) from fish fed with SPLM compared to the control diet (0% leaf meal). Meshram *et al.* (2018) reported similar results when incorporation of fermented sweet potato leaf meal in the fish diet was replaced by de-oiled rice bran in the diet of *L. rohita*. In the present study, the inclusion of sweet potato leaf meal at levels up to 100% replacement of rice bran did not reduce the growth rate and feed utilisation of *L. rohita* fingerlings and no significant difference in FCR was observed in fish fed with different levels of SPLM. Fasakin *et al.* (1999) reported that up to 30% inclusion, in the diet, duckweed (*Spirodela polyrrhiza*) supported

favourable growth in *Oreochromis niloticus*. The HSI and ISI were found similar among different treatments ($p > 0.05$) (Table 5).

Breakdown of macro nutrients into micro absorbable monomeric forms in the digestive tract of the animals depends largely on the accessible enzymes (Blier *et al.*, 2002). Ingredient source and type can alter the secretion of enzyme and its activity from the digestive tract. For better growth performance, digestive and metabolic capacity to support the tissue protein synthesis is required (Blier *et al.*, 2002), because the digestive enzymes play a key role in nutrient utilisation and growth performance of fish (Chan *et al.*, 2008, Kumar *et al.*, 2017). The breakdown of nutrients depends on the digestive enzymes activity associated with better digestion and absorption of nutrients (Kumar *et al.*, 2018b). The activity of digestive enzymes like protease, trypsin and lipase in the intestine of *L. rohita* juveniles were found similar ($p > 0.05$) among different experimental groups (Table 6). The chymotrypsin activity in the intestine was significantly ($p < 0.05$) higher in the control group (C) and lower in T3 and T4 groups. Amylase activity significantly varied among different groups and the highest amylase activity in the intestine was recorded in the T2 group ($p < 0.05$). Reduced protein digestibility may be associated with phytate and trypsin inhibitors present in SPLM (Antia *et al.*, 2006). Trypsin inhibitor inhibits the activity of protein-digesting enzymes (Ali *et al.*, 2009) and phytate forms complexes with minerals (Sugiura *et al.*, 1999) and proteins (Moyano *et al.*, 1999). In the present study, no significant growth depression was observed for sixty days of feeding trial. This observation was in corroboration with the findings of Krogdahl *et al.* (1994) and Santigosa *et al.* (2008) in trout.

Similarly, ALP activity of the liver varied significantly among treatment groups and the lowest ALP activity was observed in control group ($p < 0.05$). No significant difference was observed in lipase, protease and trypsin activities when compared with the control, but it was observed that the group fed with higher levels of SPLM had significantly lower ($p < 0.05$) chymotrypsin, ALP and amylase activity, which could be attributed to the presence

Table 5. Growth parameters of *L. rohita* fingerlings fed with different experimental diets at the end of the experiment

Treatments	Initial weight (g)	Final weight (g)	WG (%)	SGR	FCR	PER	HSI (%)	ISI (%)	Survival (%)
Control	4.30±0.03	8.06±0.55	100.58±5.01	1.11±0.11	2.01±0.29	1.65±0.28	0.77±0.05	3.18±0.26	98
T1	4.42±0.02	8.30±0.44	97.90±4.09	1.12±0.11	2.50±0.13	1.29±0.06	0.90±0.11	3.30±0.25	100
T2	4.41±0.09	9.09±0.24	106.31±5.02	1.29±0.06	2.39±0.14	1.32±0.07	0.75±0.05	4.10±0.32	95
T3	4.49±0.07	9.14±0.38	103.72±5.03	1.26±0.07	2.41±0.18	1.36±0.11	0.92±0.16	3.93±0.15	95
T4	4.49±0.03	8.56±0.21	90.42±4.45	1.29±0.04	2.74±0.13	1.14±0.05	1.04±0.12	3.51±0.36	99
p value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

WG: Weight gain; SGR: Specific growth rate; FCR: Feed conversion ratio; FER: Feed efficiency ratio; PER: Protein efficiency ratio; HSI: Hepatosomatic Index; ISI: Intestinal somatic Index. Values are Mean ± SE (n=9), Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

Table 6. Specific enzyme activities in the digestive tract of *L. rohita* fingerlings fed with different replacement level of DORB with the SPLM

Treatments	Protease (Intestine)	Trypsin (Intestine)	Chymotrypsin (Intestine)	Amylase (Intestine)	Lipase (Intestine)	ATPase		ALP	
						Liver	Intestine	Intestine	Liver
C	41.06±3.2	1.49±0.29	0.74±0.06	1.96 ^{ab} ±0.17	0.66±0.09	1.53±0.36	12.22±1.2	107.60±5.66	10.26±1.28
T1	26.65±3.3	0.73±0.16	0.49 ^b ±0.09	1.46 ^a ±0.24	0.72±0.09	1.45±0.14	18.05±2.6	61.65 ^{ab} ±6.69	15.25±3.64
T2	27.47±5.2	1.21±0.14	0.50 ^b ±0.08	2.57 ^b ±0.32	0.62±0.07	1.37 ^{ab} ±0.29	21.56±0.86	75.78 ^b ±5.65	14.37±1.64
T3	27.61±5.9	0.87±0.26	0.25 ^a ±0.03	1.80 ^a ±0.17	0.80±0.07	2.47 ^b ±0.37	18.11±2.6	46.22±2.88	11.24 ^b ±2.22
T4	26.13±4.7	0.51±0.13	0.38 ^{ab} ±0.02	1.65 ^a ±0.24	0.76±0.11	2.58 ^b ±0.27	19.22±2.8	58.43±5.73	10.72 ^b ±1.16
p value	>0.05	>0.05	<0.05	<0.05	>0.05	<0.05	>0.05	<0.05	<0.05

Activities are expressed as follows: Protease as micromole of tyrosine released per min per mg protein; Amylase as micromole unit per min per mg protein; Lipase activity as units per hour per mg protein; Alkaline phosphatase (ALP) as Nano moles p-nitrophenol released per min per mg protein at 37°C. Different superscript letters in the same column signify statistical differences (p<0.05). Data expressed as Mean ± SE (n=6)

of trypsin inhibitors and other anti-nutritional factors associated with SPLM. ALP activity of the intestine was observed to decrease with the increasing SPLM inclusion level in the diet and highest activity was observed in the control group (p<0.05) followed by T2, T1, T3 and T4 group. Alkaline phosphatase activity plays a major role in the phosphorus metabolism in the body. It catalyses the hydrolysis of various phosphate-containing compounds and acts as transphosphorylase at alkaline pH. This enzyme gets activated during the energy demand and the presence of excess levels of phosphorus in the diet. In the present study, the ALP activity in intestine and liver varied significantly. The deficiency of sodium can have an impact on the activity of Na-K-ATPase in the gastrointestinal (GI) tract and liver, which is involved in the absorption of nutrients. It has been reported that ingestion of phytic acid reduced the activity of Na-K-ATPase in the GI tract and liver in broilers (Liu *et al.*, 2008), which may be one of the reasons for the increase in the liver ATPase activity in SPLM fed groups which resulted in a reduction of phytic acid with replacement of rice bran in the diet. Replacement of DORB with SPLM significantly (p<0.05) affected ATPase activity in the liver of *L. rohita* juveniles in different experimental groups. However, ATPase activity in the intestine did not differ significantly among groups.

Based on the finding of the present study, it is concluded that SPLM can replace 100% of de-oiled rice bran in practical diets of *L. rohita*. Further, agro wastes like sweet potato leaves based feeds are cheaper as compared to the conventional feeds. However, the economic viability of incorporation of SPLM in *L. rohita* diets as a replacer of DORB needs to be studied. The present study advocates short-term utilisation efficiency but same time indicated the inevitability of long-term feeding trial with SPLM by replacing DORB and its effect on the physiology of fish. In addition to this, dietary utilisation of detoxified SPLM as a replacer of DORB can also be taken up as an approach for cost-effective use of SPLM as agriculture waste in aqua feed.

Acknowledgements

The authors are thankful to the Indian Council of Agriculture Research (ICAR), New Delhi and the Director and Vice-Chancellor, ICAR-CIFE, Mumbai, India, for providing all the facilities required for the present work.

References

- Adeolu, M. A. 2008. Potentials of sweet potato (*Ipomoea batatas*) leaf meal as the dietary ingredient for *Tilapia zilli* fingerlings. *Pak. J. Nutr.*, 7(3): 444-449. DOI: 10.3923/pjn.2008.444.449.
- Akindahunsi, A. A. and Salawu, S. O. 2005. Phytochemical screening and nutrient-anti nutrient composition of selected tropical green leafy vegetables. *Afr. J. Biotechnol.*, 4: 497-501.
- Ali, H., Haque, M. M., Chowdhury, M. M. R. and Shariful, M. I. 2009. *In vitro* protein digestibility of different feed ingredients in Thai koi (*Anabas testudineus*). *J. Bangladesh Agric. Univ.*, 7(1): 205-210. DOI: 10.3329/jbau.v7i1.4985.
- Antia, S., Akpan, E. J., Okon, P. A. and Umoren, I. U. 2006. Nutritive and anti-nutritive evaluation of sweet potato (*Ipomoea batatas*) leaves. *Pak. J. Nutr.*, 5(2): 166-168. DOI: 10.3923/pjn.2006.166.168.
- AOAC 1995. *Official methods of analysis*, 16th edn. Cunniff, P. (Ed.), Association of Official Analytical Chemists, Arlington, USA.
- APHA-AWWA-WEF 1998. *Standard methods for the examination of water and wastewater*, 20th edn. American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA.
- Bairagi, A., Ghash, K. S., Sen, S. K. and Ray, A. K. 2004. Evaluation of the nutritive value of *Leucaena leucocephala* leaf meal, inoculated with fish intestinal bacteria *Bacillus subtilis* and *Bacillus circulans* in formulated diets for rohu, *Labeo rohita* fingerlings. *Aquac. Res.*, 35: 436-446. doi.org/10.1111/j.1365-2109.2004.01028.x.
- Blier, P. U., Lemieux, H. and Devlin, R. H. 2002. Is the growth rate of fish set by digestive enzymes or metabolic

- capacity of the tissues? Insight from transgenic coho salmon. *Aquaculture*, 209(1): 379-384.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Ann. Biochem.*, 72(1-2): 248-254. doi.org/10.1016/0003-2697(76)90527-3.
- Chan, C. R., Lee, D. N., Cheng, Y. H., Hsieh, D. J. Y. and Weng, C. F. 2008. Feed deprivation and re-feeding on alterations of proteases in tilapia *Oreochromis mossambicus*. *Zool. Stud.*, 47: 207-214.
- Cherry, I. S. and Crandall, L. A. 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. *Am. J. Physiol. Legacy Content*, 100(2): 266-273. doi.org/10.1152/ajplegacy.1932.100.2.266.
- Drapeau, G. R. 1976. Protease from *Staphylococcus aureus*. *Method Enzymol.*, 45: 469-475. doi.org/10.1016/S0076-6879(76)45041-3.
- Etuk, E. U., Agaie, B. M., Ladan, M. J. and Garba, I. 2009. The modulatory effect of *Cochlospermum tinctorium* a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. *Afr. J. Pharmacol.*, 3(4): 151-157.
- Fasakin, E. A., Balogun, A. M. and Fasuru, B. E. 1999. Use of duckweed, *Spirodela polyrrhiza* Schieiden, as a protein feedstuff in practical diets for tilapia (*Oreochromis niloticus*). *Aquac. Res.*, 30: 313-318. doi.org/10.1046/j.1365-2109.1999.00318.x.
- Fiske, C. H. and Subbarow, Y. 1925. The colorimetric determination of phosphorus. *J. Biol. Chem.*, 66(2): 375-400.
- Fuller, M. F. and Chamberlain, A. G. 1982. Protein requirements of pigs. In: Haresign, W. (Ed.), *Recent advances in animal nutrition*, Butterworths, London, p. 175-186.
- Garen, A. and Levinthal, C. 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli* I. Purification and characterisation of alkaline phosphatase. *Biochim. Biophys. Acta*, 38: 470-483. doi.org/10.1016/0006-3002(60)91282-8.
- Hong, N. T. T., Wanapat, M., Wachirapakorn, C., Pakdee, P. and Rowlinson, P. 2003. Effects of timing of initial cutting and subsequent cutting on yields and chemical compositions of cassava hay and its supplementation on lactating dairy cows. *Asian Austral. J. Anim. Sci.*, 16(12): 1763-1769.
- Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Tadokoro, T. and Maekawa, A. 2000. Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potatoes (*Ipomoea batatas* Poir). *Food Chem.*, 68(3): 359-367. DOI : 10.1016/S0308-8146(99)00206-X.
- Jose, S., Mohan, M. V., Shyama, S., Ramachandran Nair, K. G. and Mathew, P. T. 2006. Effect of soybean meal based diets on the growth and survival rate of the Indian major carp, *Cirrhinus mrigala* (Ham.). *Aquac. Nutr.*, 12(4): 275-279. doi.org/10.1111/j.1365-2095.2006.00405.x.
- Krogdahl, A., Lea, T. B. and Olli, J. J. 1994. Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. Part A: Physiol.*, 107(1): 215-219. https://doi.org/10.1016/0300-9629(94)90296-8.
- Kumar, S., Sahu, N. P., Gupta, S., Deo, A. D., Shamna, N. and Ranjan, A. 2017. Inclusion level of deoiled rice bran (DORB) in the diet of *Labeo rohita* (Hamilton, 1882) fingerlings: Effect on growth and gene expression of IGF-I and IGF-II. *Aquaculture*, 481: 211-217. https://doi.org/10.1016/j.aquaculture.2017.08.025.
- Kumar, S., Sahu, N. P. and Ranjan, A. 2018a. Feeding deoiled rice bran (DORB) to Rohu, *Labeo rohita*: Effect of varying dietary protein and lipid level on growth, body composition and insulin like growth factor (IGF) expression. *Aquaculture*, 492: 59-66. https://doi.org/10.1016/j.aquaculture.2018.04.001.
- Kumar, S., Sahu, N. P., Shamna, N. and Ranjan, A. 2018b. Feeding higher level of deoiled rice bran causes stress to *Labeo rohita* fingerlings. *Aquaculture*, 484: 184-190. https://doi.org/10.1016/j.aquaculture.2017.11.029.
- Kumar, S., Sahu, N. P., Deo, A. D. and Ranjan, A. 2019. Feeding deoiled rice bran based diet with varying levels of protein and lipid: Effect on physiological responses of *Labeo rohita*. *Aquaculture*, 498: 454-463.
- Kunitz, M. 1947. Crystalline soybean trypsin inhibitor II. General properties. *J. Gen. Physiol.*, 30(4): 291-310.
- Liu, N., Ru, Y. J., Li, F. D. and Cowieson, A. J. 2008. Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. *J. Ani. Sci.*, 86(12): 3432-3439. doi: 10.2527/jas.2008-1234.
- Maity, J. and Patra, B. C. 2008. Effect of replacement of fishmeal by *Azolla* leaf meal on growth, food utilisation, pancreatic protease activity and RNA/DNA ratio in the fingerlings of *Labeo rohita* (Ham.). *Can. J. Pure Appl. Sci.*, 2(2): 323
- Meshram, S., Deo, A. D., Kumar, S., Aklakur, M. and Sahu, N. P. 2018. Replacement of deoiled rice bran by soaked and fermented sweet potato leaf meal: effect on growth performance, body composition and expression of insulin-like growth factor 1 in *Labeo rohita* (Hamilton), fingerlings. *Aquac. Res.*, 49(8): 2741-2750. https://doi.org/10.1111/are.13735.
- Moyano, F. J., Martinez, I., Daz, M. and Alarcon, F. J. 1999. Inhibition of digestive proteases by vegetable meals in three fish species; seabream (*Sparus aurata*), tilapia (*Oreochromis niloticus*) and African sole (*Solea senegalensis*). *Comp. Biochem. Physiol. Part B: Biochem. Molecul. Biol.*, 122(3): 327-332. DOI10.1016/S0305-0491(99)00024-3.
- Muin, T. A. I. H., Fatah, N. N. A., Bahari, I. H. and Razak, S. A. 2013. Rice bran replacement in *Clarias gariepinus* fingerlings diets with *Pleurotus florida* stalk. *Sains Malaysiana*, 42(8): 1109-1114.

- Ng, W. K. and Wee, K. L. 1989. The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. *Aquaculture*, 83(1-2): 45-58. [https://doi.org/10.1016/0044-8486\(89\)90059-8](https://doi.org/10.1016/0044-8486(89)90059-8).
- Oduro, I., Ellis, W. O. and Owusu, D. 2008. Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. *Scien. Res. Essays*, 3(2): 57-60.
- Post, R. L. and Sen, A. K. 1967. Sodium and potassium-stimulated ATPase. In: Estabrook, R. W. and Pullman, M. E. (Eds.), *Methods in enzymology, vol. X. Oxidation and phosphorylation*. Academic Press, New York and London, p. 762-768.
- Preston, T. R. 2006. Forages as protein sources for pigs in the tropics. *CAB Rev. : Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, 1(046): p. 1-10. <https://www.cabdirect.org/cabdirect/abstract/20063204173> (Accessed 5 August 2018).
- Ranjan, A., Sahu, N. P., Deo, A. D., Kumar, H. S., Kumar, S. and Jain, K. K. 2017. Xylanase and phytase supplementation in the De-oiled rice bran (DORB) based diet improves the growth performance of *Labeo rohita*. *Inter. J. Curr. Microbiol. Appl. Sci.*, 6(6): 1493-1503. <http://dx.doi.org/10.20546/ijcmas.2017.606.176>.
- Ranjan, A., Sahu, N. P., Deo, A. D., Kumar, H. S., Kumar, S. and Jain, K. K. 2018a. Comparative evaluation of fermented and non-fermented de-oiled rice bran with or without exogenous enzymes supplementation in the diet of *Labeo rohita* (Hamilton, 1822). *Fish Physiol. Biochem.*, 44(4): 1037-1049. doi: 10.1007/s10695-018-0492-2.
- Ranjan, A., Sahu, N. P., Deo, A. D. and Kumar, S. 2018b. Comparative growth performance, *in vivo* digestibility and enzyme activities of *Labeo rohita* fed with DORB based formulated diet and commercial carp feed. *Turk. J. Fish. Aquatic Sci.*, 18(9): 1025-1036. DOI: 10.4194/1303-2712-v18_9_02.
- Ranjan, A., Sahu, N. P., Deo, A. D. and Kumar, S. 2019. Solid state fermentation of de-oiled rice bran: Effect on *in vitro* protein digestibility, fatty acid profile and anti-nutritional factors. *Food Res. Int.*, 119: 1-5.
- Reyes, O. S. and Fermin, A. C. 2003. Terrestrial leaf meals or freshwater aquatic fern as potential feed ingredients for farmed abalone *Haliotis asinina*. *Aquac. Res.*, 34: 593-599. <https://doi.org/10.1046/j.1365-2109.2003.00846.x>.
- Riaz, M. N. 1997. Aquafeed to optimise water quality. *Feed Int.*, 18(3): 22-28.
- Rick, W. and Stegbauer, H. P. 1974. α - Amylase measurement of reducing groups. *Methods of enzymatic analysis*, 2: 885-889.
- Ruiz, M. E., Lozano, E. and Ruiz, A. 1981. Utilisation of sweet potatoes [*Ipomoea batata* (L.) Lam] in animal feeding. III. Addition of various levels of roots and urea to sweet potato forage silages. *Trop. Anim. Prod.*, 6(3): 234-244.
- Santigosa, E., Sanchez, J., Medale, F., Kaushik, S., Perez-Sanchez, J. and Gallardo, M. A. 2008. Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. *Aquaculture*, 282: 68-74. <https://doi.org/10.1016/j.aquaculture.2008.06.007>.
- Sharif, M. K. 2009. *Rice industrial byproducts management for oil extraction and value added products*, Ph. D. thesis, University of Agriculture, Faisalabad, Pakistan.
- Sugiura, S. H., Raboy, V., Young, K. A., Dong, F. M. and Hardy, R. W. 1999. Availability of phosphorus and trace elements in low-phytate varieties of barley and corn for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 170(3): 285-296. DOI: 10.1016/S0044-8486(98)00414-1.
- Tacon, A. G. J. 1993. Feed ingredients for warm water fish and other processed feed stuffs, *FAO Fisheries Circular No. 856*, Food and Agriculture Organisation, Rome, 64: 1-17.
- Tsvetanov, I. M. and Duneva, N. 1990. Study on the substitution of maize with rice bran and incineration fat in mixed feeds for broiler chickens. *Zhivotnovodni Nauki*, 27: 42-50.
- Yousif, O. M., Alhadhrami, G. A. and Passaraki, M. 1994. Evaluation of dehydrated alfalfa and Saltbush *Atriplex* leaves in diets for Tilapia (*Oreochromis aureus*). *Aquaculture*, 126: 341-347. [https://doi.org/10.1016/0044-8486\(94\)90050-7](https://doi.org/10.1016/0044-8486(94)90050-7).