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Effects of Replacing Soybean Meal with *Crassocephalum crepidioides* leaf Meal on Growth, Nutrient Utilisation and Whole Body Composition of *Labeo rohita* Fingerlings

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A B S T R A C T

The present study was conducted to evaluate the effect of soybean meal replacement with *Crassocephalum crepidioides* leaf meal (CLM) on growth, nutrient utilisation and whole body composition of *Labeorohita* fingerlings. A *C. crepidioides* leaf meal (CLM) was prepared by removing antinutritional factors through indigenous processing technique. The antinutritional factors of the processed CLM were found to decrease substantially, and the *in vitro* digestibility of the CLM was 75.31%. The nutritional potential of CLM in the diets of *Labeorohita* fingerlings (initial average weight 5.62±0.07g) were assessed in a 60 days feeding trial. Five isonitrogenous (305.0±0.08g Kg^{-1}) and isocaloric (16.74±0.02 MJ Kg^{-1}) experimental diets were formulated with a graded level of CLM, i.e. 0%, 5%, 10% or 15% in replacement for soybean meal, and designated as control, CLM5, CLM10, CLM15 respectively were fed with their respective diets to satiation twice daily at 10:00h and 18:00h. At the end of the experiment, growth performance and nutrient utilization indices such as individual weight gain (99.30-135.10%), specific growth rate (1.15-1.42%), feed conversion ratio (1.76-2.26), protein efficiency ratio (1.44-1.87) were not significantly ($p>0.05$) affected by the dietary treatments irrespective of inclusion levels of CLM. Hepatosomatic index (1.04-1.31), intestinal somatic index (4.19-4.65), survival (100%) and whole body composition of the fish among various dietary groups did not vary significantly ($p>0.05$). Thus, this study revealed that CLM is a promising alternative source of protein which could replace soybean meal up to 15% in the diets of *L. rohita* fingerlings without any adverse effects on growth, nutrient utilisation, whole body composition.

Introduction

Over the past three decades, global aquaculture production expanded at an average annual rate of more than 8%, from 5.2 million tons in 1981 to 62.7 million tons in 2011. Aquaculture's contribution to total food fish supply grew from 9% in 1980 to 48% in 2011 (FAO, 2013). Hence, a projected model of aquaculture production possible to increase from 28.6 million tons in 1997 up to 53.6 million tons by 2020 where developing countries would be responsible for 79% of world food fish production, with 77% of

global fish consumption. The assessed number of fish farmers also grew from 3.9 million in 1990 to 16.6 million in 2010. The fast and massive growth of aquaculture production has contributed significantly to the increased production of species (World Bank 2013). However, on the other side major fish feed ingredients such as soybean meal is one of the most widely used plant protein source in aqua feed production for many fish species including *Labeo rohita* (Storebakken *et al.,* 2000). Its limited availability and competition with feed production of livestock and poultry led to a rise in the price of common feed ingredients (Coffey *et al.,* 2016). Hence, there is an urgent need for alternative economically viable and sustainable aqua feed production to soybean meal.

In this regard, one of the nutritious plant, *Crassocephalum crepidioides* contains high protein value (27%) with all essential amino acids can be considered as an alternative source of protein (Dairo and Adanlawo, 2007). The *C.crepidioides* plant is locally available in North-East region of India (Worlds 12 mega biodiversity-rich zones), especially in Manipur. It is perennial herbs (Heim, 2015) and highly adaptive to harsh environments and resistance against diseases. The *Crassocephalum crepidioides* or fireweed belongs to Asteraceae family and commonly called as Terapaibee in Manipuri (Rajkumari *et al.,* 2013). A *C.crepidioides* is wild and underutilised vegetables which is a good source of micronutrients and natural antioxidants (Ng *et al.,* 2012). It is the rich source of minerals such as sodium, potassium, phosphorus, magnesium, calcium, iron, Manganese and Copper (Adjatin *et al.,* 2013).

North-east region of India is the store house of indigenous knowledge (Hanglem *et al.,* 2017). Different varieties unexplored wild of edible plants are utilised through indigenous knowledge of food preparation and preservation such as boiling, heat treatment and drying by the tribal people of Manipur (Gangte *et al.,* 2013). The contents of antinutritional factors such as phytate and saponin in *C. crepidioides*is even lesser than soybean meal. Nevertheless, cyanide contents is high in *C.crepidioides* (Etong and Abbah 2014, Hanssen 2003, Peisker, 2001). Cyanide contents in *Crassocephalum crepidioides* can be detrimental to the culture organism. So, in order to remove antinutritional factors for utilization *Crassocephalum crepidioides* leaf meal (CLM) in fish feed formulation indigenous technical knowledge (ITK) is used. Till date, no single study is available on the use of CLM in fish and livestock. Hence, with this backdrop, CLM was prepared through indigenous processing techniques and fed to *Labeo rohita* (rohu) to assess the potential utilization for aqua feed production. Due to high consumer preference, *Labeo rohita* is the most popular and widely cultured freshwater fish in South-east Asia. Thus, the present study was conducted to examine the nutritional potential of *Crassocephalum crepidioides* leaf meal (CLM) and its effect on growth performance, nutrient utilization and whole body composition of *Labeo rohita* fingerlings.

Materials and Methods

Identification and collection of herbs

The herbs were identified according to the report of Thokchom *et al.,* 2015 who described that *Crassocephalum crepidioides* S Moore is known by local name as Terapaibee, which belongs to Asteraceae family. It is wild herb found in Manipur and north east region of India. Rajkumari *et al.,* (2013) also reported that *C.crepidioides* is an edible plant species used by tribal people of Manipur for traditional medicine and other ethnobotanical purposes. The herb *Crassocephalum crepidioides* was procured from Zimthiang

village, Loktak Project, Manipur. The herb was packed in a carton box and brought to Fish Nutrition and Biochemistry Laboratory of the Central Institute of Fisheries Education (CIFE), Mumbai.

Processing/ detoxification of *Crassocephalum crepidioides* leaf meal (CLM)

Steps of CLM production and its detoxification are shown in Figure 1.

Steam blanching

Steam blanching was done by the modified method of Indriasari *et al.,* (2016). The fresh *Crassocephalum crepidioides* leaves were tied in a dry muslin cloth and placed in stainless steel cylinder with perforated side walls. The *C.crepidioides* leaves were steam blanched at 105°C for 10 minutes in auto-clave. After the blanching, the steamed *C.crepidioides* leaves were removed from auto-clave and cool down quickly to drastically reduce the temperature of the leaves in a very short duration of time and then spread into a perforated tray for air drying.

Squeezed/pressure

Squeezing of leaves was done by indigenous technical knowledge (ITK) as described by Tamang 2009. This ITK concept of pressurizing and squeezing is to remove antinutritional factors through reduction of moisture content in the leaf. The *Crassocephalum crepidioides* leaves were squeezed to remove excess water and pressed in a wide flat surface vessel.

Drying

Squeezed *Crassocephalum crepidioides* leaves were then transferred into hot air oven and dried at 60°C.

Shredding and grinding

Dried *C. crepidioides* leaves were chopped into smaller pieces and ground into *Crassocephalum crepidioides* leaf meal (CLM) in a laboratory grinder and sieved into fine meal to be used for feed formulation.

Determination of anti-nutritional factors

Cyanide

Cyanide was estimated by alkaline titration method of AOAC (1975). Around 150 ml of sample was steam-distilled into a solution of NaOH. The distillate was treated with dilute KI solution and followed by titration against 0.02 N $AgNO_3$ solution. The endpoint was obtained when there was a change from clear to a faint but permanent turbid solution. The hydrogen cyanide content was calculated by taking 1ml of 0.02 N $AgNO_3$ as equivalent to 1.08 mg Hydrogen Cyanide (HCN).

Saponin

Saponin estimation was carried out by following a gravimetric method of AOAC (1984) employing the use of a Soxhlet extractor and sequential extraction of two different organic solvents with acetone and methanol. At the end of extraction, the flask used in the extraction process was oven dried, cool in a desiccator and then weigh. Saponin content was expressed in g/kg.

Tanin

Tannin was estimated as described by Makkar *et al.,* (1993). Around 50 µL of tannins extract was taken in a test tube, and the volume made up to 1.0ml with distilled water, and then Folin Ciocalteu solution of 0.5ml was added and mixed. After mixing, 2.5ml 20% sodium carbonate solution was added and again mixed and kept for 40min at room temperature.

Optical density was taken at 725nm in spectrophotometer and results were expressed as tannic acid equivalents.

Phytic acid

Phytic acid estimation was carried out following the spectrophotometric procedure of Vaintraub and Lapteva (1998). Trichloroacetic acid (3% TCA) solution 50 mL was taken into 500 mg of sample in a flask and shaken for 30 min followed by centrifugation at 3000g for 10 min and 4 ml of ferric chloride solution was added rapidly to an aliquot of 10 mL. This was kept in a water bath at boiling temperature and centrifuged again. After washing with 3% TCA, the precipitate was dispersed in a distilled water and three mL of 1.5 N NaOH. The solution was made up to 30 mL and filtered through a Whatman No. 2 filter paper, and the precipitate was dissolved in a 40 mL hot 3.2 N nitric acid. After cooling, the volume was made to 100 mL with distilled water. From this, 5 mL aliquot was made to 100 mL using 20 mL of 1.5 M KSCN and distilled water. The reading was measured at 480 nm using a UV-visible spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan), and a blank with each sample was run. Phytic acid was expressed as percentphytic acid equivalent.

Oxalate

Oxalate was estimated according to the titration method of Day, and Underwood, 1986. 1g of a sample was added in 75ml 3M H2SO4 and stirred for 1hr with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25ml of the filtrate was titrated against warm 0.05M KMnO4 solution until a faint pink colour persisted for at least 30 sec. The oxalate content was determined by taking 1ml of 0.05m KMnO4 as equivalent to 2.2mg oxalate (Chinma, & Igyor 2007; Ihekoronye and Ngoddy 1985).

In vitro protein digestibility

In vitro protein digestibility study was done as per the procedure of Ali *et al.,* (2009). A fresh tissue of the alimentary canal was homogenized under cold condition and diluted with distilled water (1:10 w/v). Enzyme was extracted by centrifuging it at 12000 rpm for 15 min at 4 °C. An equivalent amount of finely *ground C. crepidioides* lm that provided 160 mg of crude protein was weighed and mixed with 20 mL of distilled water and 2 mL of the enzyme to obtain 8 mg crude protein per millilitre and the pH was adjusted to 8 (Eutop pH tutor, Thermo Fisher Scientific, Singapore). The pH drop was recorded at every minute interval for 10 min, and casein was used as the reference protein. Relative Protein Digestibility was estimated using the following formula

Relative Protein Digestibility (RPD %) = (-ΔpH of ingredients/-ΔpH of casein) x 100.

Proximate analysis

Proximate analysis of *Crassocephalum crepidioides* leaf meal (CLM) and feed (on dry matter basis) and muscle tissue (on wet weight basis) were performed as per the standard method of AOAC (1995). Digestible energy was calculated using the following formula:

Digestible energy (DE, MJ Kg^{-1}): [16.74 × CP (g Kg^{-1}) + 37.66 × EE (g Kg^{-1}) +16.74 × TC (g Kg^{-1})]/1000 (Harvel 1976)

Experimental diets

The experimental diets were divided into four groups which were isonitrogenous (305.0±0.08 g Kg^{-1}) and isocaloric (16.74±0.02 MJ Kg^{-1}). The soybean meal was replaced at 0%, 5%, 10% or 15% with *Crassocephalum crepidioides* leaf meal

(CLM) which was designated as Control, CLM5, CLM10, CLM15, respectively (Table 1). The ingredients were ground and mixed thoroughly to form a homogenous blend followed by addition of vitamin-minerals mixture, oil and water to form a dough. The prepared dough was passed through a pelletizer using 2mm die and the pellets were air dried, and stored at -20 °C until further use.

Acclimatisation of fish and experimental setup

Fingerlings of Indian major carp, *Labeo rohita* ranging between 5.27g and 6.13g body weight were procured from Arey fish farm, Goregaon, Mumbai, India.

The fishes were transported in a big circular container (500 L) with sufficient aeration to the wet laboratory of Central Institute of Fisheries Education (CIFE). The fishes were given a mild salt dip treatment (20 g L^{-1}) for 2 min before transferring to another circular tank (1000 L). The stock was acclimatized under aerated conditions in the same circular tank for a period of 15 days.

The experiment was conducted in 12 plastic rectangular tubs (75L capacity) covered with perforated lids previously treated and cleaned with potassium permanganate (KMnO4) solution.

One hundred and forty-four fingerlings were randomly distributed in four distinct experimental groups. The experiment was conducted for a period of 60 days and fishes were fed at 3% of the body weight. The daily amount of feed was section into two equal parts and was fed at 10:00 and 18:00h using the respective experimental diets. Uneaten feed, together with feces, was carefully siphoned out manually. Water quality was monitored throughout the experiment (APHA 1998).

Fish sampling

At the end of feeding trial the fishes were starved overnight and then weighed for calculating the growth performance and nutrient utilization parameters such as weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER). Fishes were sampled from each replicate and anaesthetized, tissues of different organs liver and intestine were dissected out. Body indices parameters like hepato-somatic index and intestine-somatic index were calculated. For proximate analysis, all the dissected fishes from every replicate were collected, weighed and kept in pre-weighed Petri plates.

Calculations

Following parameters related to growth and nutrient utilization were calculated using standard formula.

Weight gain (%) = [(final weight-initial weight)/initial weight] x 100; specific growth rate (SGR, %) = 100 x (ln final body weight-ln initial body weight)/experimental duration in days; feed conversion ratio (FCR) ={feed consumption (g on dry weight basis)/body weight gain (g on wet weight basis)}; protein efficiency ratio (PER) ={net weight gain (g on wet weight basis)/protein fed (g on dry matter basis)} and the survival (%) = [(Total number of fish harvested/ total number of fish stocked) x 100]. Hepatosomatic index (HSI) and intestinal somatic index (ISI) were calculated using the following formula:

$$HSI\ (\%) = \frac{\text{Weight of liver (g)}}{\text{Weight of fish (g)}} \times 100$$

The gastrointestinal tract of different treatment groups were recorded and the gastrointestinal index was calculated as follows

$$ISI\ (\%) = \frac{\text{Weight of intestine (g)}}{\text{Weight of fish (g)}} \times 100$$

Statistical analysis

Data were statistically analyzed by SPSS package version 16.0 which were subjected to one way ANOVA and Duncan's multiple range test to determine the significant differences between the means. Comparisons were made at the 5% probability level.

Results and Discussion

Proximate composition of *Crassocephalum crepidioides* leaf meal (CLM) and experimental diet

The results of proximate composition of *Crassocephalum crepidioides* leaf meal (CLM) and the experimental diet are presented in Table 2. The proximate composition of *C.crepidioides* leaf meal (CLM) viz, crude protein (g Kg^{-1}) ranges from 268.9 to 276.3, crude lipid (g Kg^{-1}) ranges from 26.5 to 30.4, ash (g Kg^{-1}) level varies from 186.4 to 194.8, and digestible energy (MJ Kg^{-1}) ranges from 14.03-14.24. On the other side, the proximate composition of the experimental diet showed crude protein (g Kg^{-1}) ranges between 301.6 to 310.2, crude lipid (g Kg^{-1}) varies from 61.8-72.4, nitrogen-free extract (g Kg^{-1}) varies from 467.8-480.3, crude fibre (g Kg^{-1}) ranges from 62.8-75.3, ash (g Kg^{-1}) ranges from 80.5-89.6, digestible energy (MJ Kg^{-1}) levels was in between 16.60-16.82.

Antinutritional factors of unprocessed *C. crepidioides* leaf meal and processed *C. crepidioides* leaf meal (CLM)

The results of antinutritional factors of unprocessed *C. crepidioides* leaf meal and processed *C. crepidioides* leaf meal (CLM) are presented in Table 3. Antinutritional factors present in *C. crepidioides* leaf meal are cyanide, phytic acid, saponin, oxalate and tannin. Cyanide was removed to maximum extend from 11.85 mg HCN Kg in unprocessed *C. crepidioides* leaf meal to 2.83 mg HCN Kg in processed *C. crepidioides* leaf meal (CLM).

In vitro protein digestibility and water quality parameters

The result of protein digestibility of *Crassocephalum crepidioides* leaf meal (CLM) in *in vitro* study was found to be 75.31%.

Water quality parameters are given in Table 4. The water quality parameters such as temperature was 24.8-28.5°C, dissolve oxygen 5.6-7.1 mg/L, pH 7.2-8.3 and ammonia 0.01-0.06 mg/L.

Growth performance, nutrient utilisation, hepatosomatic index (HSI), intestinal somatic Index (ISI) and survival

Studies on growth and nutrient utilisation of the fish were exhibited in terms of the weight gain (%), SGR, FCR, PER, HSI and ISI. Higher weight gain, SGR, PER and lower FCR were found in the control group, CLM5 and CLM10 compare to CLM5.

However weight gain (%), SGR, FCR, PER, HIS, ISI and survival of the fish among different experimental groups were not affected significantly ($p>0.05$) through the feeding of CLM (Table 5).

Whole body composition of the fish

Whole body composition was presented in Table 6. It was observed that feeding of CLM did not show any significant trend in the whole body composition of fish in the experimental groups.

Fig.1 Process of *Crassocephalum crepidioides* leaf meal production and its detoxification

Raw leaves of *Crassocephalum crepidioides*
↓
Steam blanching
↓
Squeezing/pressurizing
↓
Drying
↓
Shredding and grinding.

Table.1 Composition of the test diets used during experimental feeding trial (g Kg^{-1})

Ingredients (g Kg^{-1})	Treatments			
	Control	CLM5	CLM10	CLM15
Soybean meal	210	200	189	178.5
***Crassocephalum crepidioides* leaf meal (CLM)**	0	10.5	21	31.5
Fish meal	60	60	60	160
Mustard oil cake	169.7	170	170	170
Ground nut oil cake	200	210	210	220
Wheat flour	150	150	150	150
Rice bran	118.3	107.5	108	98
Fish oil	20	20	20	20
Sunflower oil	40	40	40	40
Vitamin/mineral mix	20	20	20	20
Carboxymethyl cellulose	10	10	10	10
Butylatedhydroxytoluene	2	2	2	2

Composition of vitamin mineral mix (PREEMIX PLUS, Himedia, India) (quantity/2.5kg), Vitamin A, 55,00,000 IU; Vitamin D_3, 11,00,000 IU; Vitamin B_2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B_6, 1,000 mg; Vitamin B_{12}, 6 mcg; 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, 50 ppm

Table.2 Proximate composition (g Kg^{-1} dry matter basis) of *Crassocephalum crepidioides* leaf meal (CLM) and experimental diets

Variables	*C. crepidioides* leaf meal (CLM)	Experimental diets			
		Control	CLM5	CLM10	CLM15
Crude protein	272.9±0.21	304.5±0.13	304.1±0.15	305.1±0.22	306.3±0.22
Crude lipid	28.8±0.12	70.7±0.08	68.0±0.18	65.2±0.13	66.5±0.25
Nitrogen free extract (NFE)	416.8±0.25	472.9±0.37	473.8±0.24	473±0.24	472.0±0.11
Crude fibre	90.1±0.22	65.5±0.19	68.7±0.20	70.7±0.11	73.0±0.11
Ash	191.2±0.24	86.4±0.15	84.9±0.12	87.2±0.14	82.0±0.09
Digestibleenergy (MJ Kg^{-1})	14.14±0.06	16.78±0.03	16.73±0.01	16.67±0.04	16.76±0.05

Table.3 Anti-nutritional factors of unprocessed *Crassocephalum crepidioides* leaf meal and processed *Crassocephalum crepidioides* leaf meal (CLM)

Anti-nutritional factors	Unprocessed *C. crepidioides* leaf meal	Processed/detoxified *C. crepidioides* leaf meal (CLM)
Cyanide (mgHCN $Kg^{-1)}$	11.85	2.83
Phytic acid (g $Kg^{-1)}$	2.14	1.13
Saponin (g $Kg^{-1)}$	0.43	0.19
Oxalate (g $Kg^{-1)}$	0.30	0.17
Tannin (g $Kg^{-1)}$	0.09	0.03

Table.4 Physico-chemical parameters of water during the experimental period of 60 days for different experimental groups

Sl. No.	Parameters	Ranges
1.	Temperature	24.8-28.5°C
2.	pH	7.2- 8.3
3	Dissolved oxygen	5.6-7.1mg/L
4	Total hardness	137-198mg/L
5	Ammonia	0.01-0.06mg/L
6.	Nitrite	0.008-0.02mg/L
7.	Nitrate	0.96-1.5mg/L

Table.5 Growth performance, nutrient utilization, survival of *Labeo rohita* fingerlings fed with different experimental diets

Treatment	Parameters						
	Weight gain (%)	SGR (%)	FCR	PER	Survival (%)	HSI	ISI
Control	128.79±3.11	1.38±0.02	1.85±0.04	1.78±0.04	100	1.15±0.07	4.36±0.03
CLM5	124.02±5.69	1.34±0.04	1.92±0.06	1.71±0.05	100	1.21±0.03	4.40±0.09
CLM10	116.74±4.67	1.29±0.03	1.96±0.04	1.67±0.02	100	1.19±0.02	4.51±0.10
CLM15	114.27±7.58	1.26±0.06	2.04±0.11	1.61±0.08	100	1.13±0.08	4.29±0.08

Values in the same column were not significantly different (P<0.05). Data expressed as mean ±SE (n=3)
SGR (%): Specific growth rate, FCR: Feed conversion ratio, PER: Protein efficiency ratio,
HSI: Hepatosomatic index, ISI: Intestinal somatic index

Table.6 Proximate composition of the whole body of *Labeo rohita* fingerlings of different experimental groups (% wet wt. basis ±SE)

Variables	Experimental groups			
	Control	CLM5	CLM10	CLM15
Moisture	73.94±0.19	73.24±16	73.56±18	73.76±20
Crude protein	15.87±20	15.75±21	15.84±23	15.60±19
Crude lipid	5.51±19	5.85±22	5.48±19	5.86±27
Ash	2.80±13	3.05±25	2.56±21	2.48±18

Values in the same row were not significantly different (P<0.05). Data expressed as mean ±SE (n=3)

Certain underexploited nutritious plant can be utilised effectively once the presence of anti-nutritional factors is removed. Various methods of blanching, squeezed, drying to reduce anti-nutritional factors of *Crassocephalum crepidioides* (Nupo *et al.*, 2013). Steaming, sun-drying, shredding reduce cyanide and phytate in cassava leaves (Abok *et al.*, 2016, Montagnac *et al.*, 2008). In the present study, *Crassocephalum crepidioides* leaf meal (CLM) was treated with step by step detoxification process such as steam blanching, squeezing, drying, shredding/gringding and the results obtained after analysis showed that the amount of antinutritional factor present in CLM is detoxified to safe level which can be tolerated by a monogastric animal including human. This is comparable with the report of (Nupo *et al.*, 2013, Ilelaboye *et al.*, 2013) which proved *Crassocephalum crepidioides* can be detoxified to a safe level. Tagwireyi *et al.*, (2008) also reported that steamed treated diets showed better growth performance than boiled diets in Nile tilapia fry.

The *in vitro* protein digestibility of CLM was 75.31% which was higher than the *in vitro* digestibility of cotton seed cake and rubber seed cake in *Labeo rohita* 73.61% and 66.54% respectively (Hasnat *et al.*, 2015). Ali *et al.*, 2009 also reported that *in vitro* digestibility of soybean meal was 79.41% in *Puntiusgonionotus*. A feeding trial was conducted on *Labeo rohita* fingerlings feed with processed/detoxified *Crassocephalum crepidioides* leaf meal (CLM). All the physical-chemical parameters in the water remained within the range recommended for fish culture (Boyd, 1990) which suggests that water quality do not cause any physiological stress to the fish.

In the present study, no significant variation was observed in the growth performance of the fish fed CLM in replacement for soybean meal. The lack of differences in the PER and FCR indicate that the CLM was well digested and utilized by the fish. This observation showed a good congruence with recent studies of Tiamiyu *et al.,* (2016) who reported that Moringa leaf meal can substitute 50% of soybean meal in the diet of *Oreochromisniloticus* without affecting the growth and nutrient utilisation. Kasiga *et al.,* (2014) also observed no significant difference in *Oreochromisniloticus* fed *Moringaoleifera* leaf meal or *Leucaenaleucocephala* leaf meal replacing up to 30% of the soybean meal protein despite lower nutrient availability compared with soy diet. Similar results were also shown by Mohapatra *et al.,* (2015) that a diet consisting of *Eichhornia crassipes* meal up to 40% content could be used as a replacement for fish meal in diet formulation for common carp fry (Mohapatra 2015).

The presence of anti-nutritional factors in plant-based diets is one of the reasons for the reduction of feed intake, nutrient absorption and growth retardation in fish due to unpleasant tastes and poor feed acceptability (Francis and Becker, 2001). However, the inclusion of CLM in the diets of the fish in this study did not cause any significant difference in the whole body composition and survival of the fish in various treatments groups. This is in agreement with Hussein *et al.,* (2016) who reported that whole body composition and survival of Nile Tilapia were not affected by the dietary replacement of yellow corn with sorghum meal.

The HIS and ISI values did not differ significantly among the fish of different experimental groups, which is in agreement with the study of Mishra *et al.,* (2017), who observed that *L. rohita* fed *Westleopsis prolifica*, algae as a major dietary ingredients showed no significant differences in HSI. Phulia *et al.,* (2017) also found no significant differences in HSI and ISI of *L. rohita* fed fermented Jatropha kernel meal in replacement of soybean meal. This lack of differences in the HSI and ISI indicate that the physiological functions and survival of fish were not compromised as a result of feeding CLM.

Based on the observations in the present study, it is revealed that CLM is a rich plant protein source. Processed or detoxified CLM showed a considerable value of *in-vitro* digestibility and no significant reduction in the feed consumption of *L. rohita*. Feeding processed or detoxified CLM upto 15% replacement of soybean meal showed improvement of the fish growth, no significant mortality and whole body composition suggesting its potential use in aquafeed. Therefore, CLM could replace possibly up to 15% of soybean without any detrimental effect in growth and survival of the fish and become a promising alternative plant protein source in search for sustainable and economically viable ingredients for aquafeed industries.

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