

Substitution of soybean meal with fermented *Jatropha* kernel meal: effect on growth performance, body composition, and metabolic enzyme activity of *Labeo rohita*

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Received: 1 May 2017 / Accepted: 22 November 2017
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Abstract A 60-day feeding trial was conducted to evaluate the nutritional potential of fermented *Jatropha* kernel meal (FJKM) in the diets of rohu, *Labeo rohita*, fingerlings. Four iso-nitrogenous (336 g kg^{-1}) and iso-energetic (20 MJ kg^{-1}) diets containing 0, 100, 200, and 300 g kg^{-1} FJKM in replacement of 0, 33.3, 66.7, and 100% soybean meal protein, respectively, were prepared and designated as control, T1, T2, and T3, respectively. A total of 180 acclimatized fish (average weight $6.05 \pm 0.04 \text{ g}$) were equally distributed into four experimental groups in triplicates, and were fed their respective diets to satiation twice daily at 10:00 h and 18:00 h. Higher percent weight gain (%WG); specific growth rate (SGR); protein efficiency ratio (PER); protease, aspartate amino transferase (AST),

and alanine amino transferase (ALT) activities; and lower feed conversion ratio (FCR) were recorded in T2 and T3 groups compared to other groups. Whereas hepatosomatic index (HSI), intestinal somatic index (ISI), and amylase, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), superoxide dismutase (SOD), and catalase activities among the various dietary groups did not vary significantly ($P > 0.05$). Whole body composition of fish analyzed at the end of the feeding trial exhibited significantly ($P < 0.05$) higher ether extract and lower crude protein in the control group compared to the FJKM-fed groups. These results indicated that rohu fingerlings can efficiently utilize FJKM without any detrimental effect on growth performance, nutrient utilization, and metabolic response.

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Keywords Fermentation · *Jatropha* kernel meal ·
Growth · Nutrient utilization · Metabolic enzymes · Body
composition

Introduction

The aquaculture sector is progressing at an annual growth rate of about 8.6% (FAO 2014) and is currently focused as the fastest-growing sector compared to all other terrestrial farmed animal production systems (Troell et al. 2014). By the year 2030, 121.6 metric tons (MT) of additional fish would be required from global aquaculture to meet the projected demand of 183 MT with 22.5 kg per capita

fish consumption (Brugere and Ridler 2004). This would be possible only with further expansion and intensification of aquaculture which is often associated with an increased demand for aquafeeds. Over the years, there has been a manifold increase in the price of aquaculture feedstuffs, but the farm gate price of cultured fish has not shown a proportionate increase. This phenomenon has limited further expansion and profitability of aquaculture enterprise especially in developing countries. Traditionally, fish meal (FM) has been the major dietary protein source used for fish feed preparation due to its excellent nutritional value (Kumar et al. 2011), but an almost stagnant marine fish production level concurrent with high market price of fish meal has stimulated several studies exploring the partial or complete replacement of FM by several plant protein sources (Kaushik et al. 1995; Fournier et al. 2004). Soybean meal (SBM) is a potential alternative to FM and is currently the most preferably used plant protein source in fish feeds owing to its outstanding nutritional makeup (Yue and Zhou 2009). Since 2005, its basket commodity price index (CPI) has increased by about 50% and the price of SBM raised by about 67% (Rana et al. 2009). Based on the current supply and demand forecasts for the coming years, prices can be expected to remain high (Kumar et al. 2010). In addition, there is a competition in both human and terrestrial animal sectors for soybean as food or feed ingredient (Kumar et al. 2011; Fawole et al. 2016a, b). This has necessitated a search for some other alternative plant protein sources in fish feed.

Jatropha curcas (L.), which is commonly known as physic nut, is a multipurpose and drought-resistant tree, widespread throughout the tropics and subtropics (Makkar and Becker 2009). It is a hardy plant which thrives well on degraded land and requires limited amounts of nutrients and water (Singh et al. 2008). Improved *Jatropha* plant can yield 4 to 5 t seed per year from 1 ha of plantation (Heller 1996). The seed kernels have been reported to contain about 60% oil that can be trans-esterified to biodiesel (Azam et al. 2005), and the kernel meal which is a by-product after oil extraction is an excellent source of protein (58 to 62% crude protein) for animal (Makkar et al. 2007a, 2008). One ton of seeds yield 270 kg of *Jatropha* kernel meal (Makkar and Becker 2009; Guedes et al. 2014). By

2020, approximately 7% of the global biodiesel production, i.e., 2.94 MT, is expected to come from *J. curcas* (OECD/FAO 2011) generating approximately 2.76 MT of kernel meal and 7.35 MT of seed cake (Makkar 2016). Despite having a high protein content and an amino acid profile superior to SBM (except lysine), incorporation of *Jatropha* kernel meal in fish feed largely remains restricted due to the presence of phorbol esters (PEs), a major toxic component (Makkar and Becker 1997, 1998), and other antinutritional factors such as trypsin inhibitor, phytate, lectin, and tannin (Makkar et al. 2007b, 2008). Several methods including solvent extraction (using organic solvents, e.g., methanol and ethanol), chemical treatment (using alkali, e.g., NaOH), and biological treatment (using fungi and bacteria) (Rakshit et al. 2008; Makkar and Becker 2010; Joshi et al. 2011; Shamna et al. 2015; Hassaan et al. 2016; Phulia et al. 2017) have been devised to remove the toxic components/antinutritional factors (ANFs) for effective utilization of *Jatropha* kernel meal in fish feed. Use of biological methods such as solid state fermentation (SSF) for detoxification of *Jatropha* kernel meal, however, is still in the nascent stage (Hassaan et al. 2016). SSF constitutes a fermentation process performed on a non-soluble material that acts as both physical support and source of nutrients in the absence of free-flowing liquid (Pandey 1992). In addition to removal of ANFs, fermentation of SBM has also been reported to improve nutritional quality of sunflower meal (Canella et al. 1984), amino acid composition of *Jatropha* seed meal (Hassaan et al. 2016), and digestibility of SBM (Kiers et al. 2000). There are very few studies which have evaluated effects of fermented *Jatropha* products on growth performance, nutrient digestion efficiency, and physiological status of cultivable fish species, especially for *Labeo rohita* (Saha and Ghosh 2013; Shamna et al. 2015, 2016; Phulia et al. 2017) and *Oreochromis niloticus* (Hassaan et al. 2016). Due to high consumer preference, *Labeo rohita* is the most important among the three Indian major carp species used in carp polyculture systems. With this backdrop, the present study planned to evaluate the effect of fermented *Jatropha* kernel meal (FJKM) in replacement for SBM on the growth performance, nutrient utilization, body composition, and digestive

and metabolic enzyme activities in *Labeo rohita* fingerlings.

Materials and methods

Preparation of *Jatropha* kernel meal

Jatropha curcas seeds procured from Genius Herbs, Coimbatore, were dehulled manually to obtain the kernels. The kernels were ground with the help of a kitchen grinder and defatted using diethyl ether in a Soxhlet extraction apparatus. The defatted *Jatropha* kernel meal (DJKM) was oven dried at low temperature (50 °C), cooled, packed in airtight polythene bags, and stored at −20 °C until use.

Solid state fermentation of DJKM

The *Aspergillus niger* (MTCC 281) used for the SSF was procured from IMTECH, Chandigarh, India, and SSF was done following the method described by Belewu and Sam (2010).

The subculture of fungi was developed using Czapek Yeast Extract Agar (CYA) as suggested by IMTECH. The prepared CYA was autoclaved and poured in sterilized dishes and kept overnight. On the next day, plates were checked for contamination, if any. Uncontaminated plates were inoculated with *A. niger* for fungal growth. Once fungus grew fully on CYA plates, fungal spores were harvested using Tween 80 solution and adjusted to 10^7 – 10^8 spores per milliliter by a serial dilution method. Fermentation of *Jatropha* kernel meal was carried out in a wide-mouth sterile conical flask and conducted over a period of 3 weeks. After the fermentation period was over, further fungal growth was terminated by autoclaving. The fermented product was lyophilized, designated as fermented *Jatropha* kernel meal (FJKM), and stored at −20 °C until use.

Estimation of toxic principle and antinutritional factors

Phorbol esters

The phorbol esters (PEs) were extracted from the detoxified *Jatropha* kernel meal following the method of Saetae and Suntornsuk (2010). Twenty milliliters of methanol was added to 5 g of sample and stirred on an

orbital shaker at 200 rpm for 5 min. The content was filtered using a Whatman No.1 filter paper, and the filtrate was collected. The extraction was repeated four times. The entire extraction fraction was pooled and concentrated in a rotary evaporator (IKA RV 10, Germany). The PE was quantified in HPLC using the method of Makkar et al. (2007b) and expressed as milligrams per gram.

Phytic acid

Phytic acid estimation was carried out following the spectrophotometric procedure of Vaintraub and Lapteva (1998). Five hundred milligrams of finely ground sample was taken in an Erlenmeyer flask, and 50 mL 3% trichloroacetic acid (TCA) solution was added. After shaking for 30 min, the mixture was centrifuged at 3000g for 10 min. Four milliliters of ferric chloride solution was added rapidly to an aliquot of 10 mL. This was kept in a water bath at boiling temperature for 45 min. The solution was centrifuged at 3000g for 10–15 min. After washing with 3% TCA, the precipitate was dispersed in few a milliliters of distilled water and 3 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 100-mL volumetric flask containing 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 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 percent phytic acid equivalent.

Trypsin inhibitor

Trypsin inhibitor was estimated by following the modified method of Makkar et al. (2007b). One gram of finely ground sample was mixed with 50 mL of 0.01 M NaOH and was stirred for 3 h at room temperature at a pH range of 8.4 to 10. Aliquots (0, 0.6, 1.4, and 1.8 mL) of suspensions were pipetted and made up to 2.0 mL with distilled water. Two milliliters of trypsin (Sigma, St. Louis, USA) solution was added, and the tube was placed in a water bath at 37 °C for 10 min. The reaction was terminated by adding 1 mL of 30% acetic acid. The contents were centrifuged, and the absorbance was

measured at 410 nm using a UV-visible spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan) against the reagent blank. The activity was expressed in trypsin inhibitor units (TIU).

Tannin

Total tannin was estimated using the spectrophotometric method as described by Makkar et al. (2007b). The reagent used was the Folin–Ciocalteu reagent, and the results were expressed as tannic acid equivalents.

Formulation and preparation of experimental diets

Four iso-nitrogenous (336 g kg⁻¹) and iso-energetic (20 MJ kg⁻¹) diets containing 0, 100, 200, and 300 g kg⁻¹ FJKM in replacement of 0, 33.3, 66.7, and 100% of SBM protein were formulated and designated as control, T1, T2, and T3, respectively (Table 1). SBM, FJKM, FM, and GNOC were used as protein source, maize flour, wheat bran, and rice flour were used as carbohydrate source, sunflower oil and cod liver oil were used as lipid source, CMC was

Table 1 Feed and proximate composition of the experimental diets (DM basis) fed to *Labeo rohita* fingerlings during the 60-day experimental period

Ingredients	Treatment ^a			
	Control	T1	T2	T3
SBM	300	200	100	0
FJKM	0	100	200	300
FM	50	50	50	50
GNOC	260	240	220	200
Maize flour	100	100	120	140
Wheat bran	119.8	139.8	139.8	139.8
Rice flour	80	80	80	80
Sunflower oil	30	30	30	30
Cod liver oil	30	30	30	30
CMC	10	10	10	10
BHT	0.2	0.2	0.2	0.2
Vitamin and mineral mix	20	20	20	20
Total	1000	1000	1000	1000
Proximate composition (g kg ⁻¹)				
Moisture	64.0 ± 0.4	66.3 ± 1.2	63.8 ± 0.3	64.2 ± 1.7
CP	338.1 ± 1.7	337.2 ± 2.1	339.0 ± 2.7	332.5 ± 5.3
EE	87.5 ± 5.3	93.0 ± 3.4	97.3 ± 3.1	95.1 ± 5.1
TA	71.6 ± 4.2	75.5 ± 3.7	77.0 ± 3.5	79.5 ± 4.0
CF	72.0 ± 1.5	76.3 ± 2.1	79.4 ± 2.6	75.1 ± 1.3
NFE	430.8 ± 7.2	418.0 ± 3.2	407.3 ± 4.1	417.8 ± 4.5
GE (MJ kg ⁻¹)	20.09 ± 0.9	20.14 ± 0.6	20.23 ± 0.6	20.09 ± 1.2

Data expressed as mean ± SE, *n* = 3. Composition of vitamin mineral mix (Premix Plus) (quantity 2.5 kg⁻¹): vitamin A, 55,00,000 IU; vitamin D3, 11,00,000 IU; vitamin B2, 2000 mg; vitamin E, 750 mg; vitamin K, 1000 mg; vitamin B6, 1000 mg; vitamin B12, 6 mcg; calcium pantothenate, 2500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 L-lysine, 10 g; DL-methionine, 10 g; selenium, 125 mg; vitamin C, 2500 mg. Gross energy (MJ kg⁻¹) = [19 × CP (g kg⁻¹) + 36 × EE (g kg⁻¹) + 15 × TC (g kg⁻¹)] kJ kg⁻¹ 1000⁻¹ (Smith 1981)

SBM soybean meal, FJKM fermented *Jatropha* kernel meal, FM fish meal, GNOC ground nut oil cake, CMC carboxymethyl cellulose, BHT butylated hydroxytoluene, CP crude protein, EE ether extract, TA total ash, CF crude fiber, NFE nitrogen-free extract, GE gross energy

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

used as pellet binder, butylated hydroxytoluene (BHT) was used as antioxidant preservative, and vitamin–mineral mixture was used as feed supplement in the formula. Ground ingredients were mixed properly, and a dough was prepared with the addition of an adequate quantity of water followed by cooking in a pressure cooker for half an hour. The vitamin–mineral mixture and BHT were added to the dough after cooling. The dough was pressed through a pelletizer with 2 mm diameter. Finally, the feed pellets were air dried, followed by drying in a hot air oven at 60 °C until the moisture level was less than 12%. The dried experimental diets were packed in airtight polythene zipper bags and stored at 4 °C until use.

Amino acid profile in DJKM, FJKM, and SBM

The amino acid profile except tryptophan of DJKM, FJKM, and SBM was determined using an automated amino acid analyzer after hydrolyzing the samples with 6 M HCl at 110 °C for 24 h (Ishida et al. 1981). The tryptophan content of the abovementioned samples was quantified spectrophotometrically by the method of Sastry and Tammuru (1985).

Experimental system and fish maintenance

Fingerlings of Indian major carp, *L. rohita*, with body weight ranging from 5.81 to 6.30 g were procured from Arrey Fish Farm, Goregaon, Mumbai. The fish were transported in a big circular container (500 L) with sufficient aeration to the central wet laboratory, ICAR-Central Institute of Fisheries Education, Mumbai, India. The fish were given a dip treatment in mild salt solution (20 g L⁻¹) for 2 min before transferring them to another circular tank (1000 L) and were left undisturbed for the whole night. Fishes were acclimatized to experimental rearing conditions for 15 days and fed with control diet. The experiment was carried out over a period of 60 days. One hundred eighty acclimatized fish (average weight 6.05 ± 0.04 g) were randomly distributed into 12 experimental tubs of 300-L capacity with 15 fish per tub following a complete

randomized design (CRD). Under each treatment group, three replicates were maintained. Round-the-clock aeration was provided throughout the experimental period. Fishes were fed their respective experimental diets daily to satiation level at 10:00 h and 18:00 h. Fecal matter was siphoned out carefully. Water quality was monitored throughout the experimental period (APHA 1998).

Fish sampling

At the end of the experiment, the fish were starved overnight before taking the final body weight. Three fish were sampled from each replicate and anesthetized using clove oil (50 µL of clove oil per liter of water). Tissues of different organs (muscle, liver, and intestine) were dissected out and homogenized with 0.25 M chilled sucrose solution in glass tubes using a Teflon-coated mechanical tissue homogenizer (MICCRA D-9, ART Prozess- & Labortechnik, Germany). The tubes were continuously kept in ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 min at 4 °C (REMI CPR-24, India). The supernatant was collected and stored at -20 °C until use. A 5% homogenate was prepared for the tissues of interest for enzyme assay. Liver and intestine from another three fish were pooled together, and weight was taken for calculation of hepatosomatic index (HSI) and intestinal somatic index (ISI), respectively. Three fish from each experimental group were subjected to analysis of whole body composition.

Proximate analysis of diets and fish

The proximate composition of the experimental diets (on dry matter basis) and fish (on wet weight basis) was determined using the standard methods of the Association of Official Analytical Chemists (AOAC 1995). Samples were analyzed for moisture, dry matter, crude protein, lipid, total ash, crude fiber, and nitrogen-free extract. Gross energy (GE) of diets was calculated using the formula given by Smith (1981):

$$\text{GE (MJ kg}^{-1}\text{)} = [19 \times \text{CP (g kg}^{-1}\text{)} + 36 \times \text{EE (g kg}^{-1}\text{)} + 15 \times \text{TC (g kg}^{-1}\text{)}] \text{ kJ kg}^{-1} \times 1000^{-1}$$

Calculation for growth performance, nutrient utilization, and survival

Growth performance and nutrient utilization were assessed in terms of percent weight gain (%WG),

$$\begin{aligned}\%WG &= [(final\ weight - initial\ weight) / initial\ weight] \times 100; FCR \\ &= \{feed\ consumption\ (in\ g\ on\ dry\ weight\ basis) / body\ weight\ gain\ (in\ g\ on\ wet\ weight\ basis)\}; PER \\ &= \{net\ weight\ gain\ (in\ g\ on\ wet\ weight\ basis) / protein\ fed\ (in\ g\ on\ dry\ matter\ basis)\} \text{ and survival } (\%) \\ &= [(total\ number\ of\ fish\ harvested / total\ number\ of\ fish\ stocked) \times 100].\end{aligned}$$

Hepatosomatic index and intestinal somatic index

Hepatosomatic index and intestinal somatic index were calculated using the following formula:

$$HSI (\%) = [wet\ weight\ of\ liver\ (g) / wet\ weight\ of\ fish\ (g) \times 100]$$

$$ISI (\%) = [wet\ weight\ of\ intestine\ (g) / wet\ weight\ of\ fish\ (g) \times 100]$$

Digestive and metabolic enzymes activity assay

Quantification of protein in the intestinal tissues was carried out using Lowry's method (Lowry et al. 1951). Tissue homogenate (0.1 mL) was taken in a test tube and precipitated using 1 mL of 10% TCA followed by centrifugation at 5000 rpm for 20 min to collect the protein, which was dissolved in 0.5 mL of 0.1 N NaOH. Alkaline copper sulfate (5 mL) was added to 0.1 mL of the dissolved protein residue and left for 10 min. To this, 0.5 mL of 1 N Folin's reagent was added and incubated for 30 min in dark conditions. OD was recorded at 660 nm against the blank. Bovine serum albumin (BSA) was used as a standard.

Amylase activity was estimated as the reducing sugars produced due to the action of glucoamylase and α -amylase on carbohydrate using the dinitrosalicylic acid (DNS) method (Rick and Stegbauer 1974). Specific amylase activity was expressed as mole of maltose released from starch per minute per milligram of protein at 37 °C. Specific protease activity was determined by the casein digestion method (Drapeau 1974). One unit

specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER). These were calculated as follows:

of enzyme activity was defined as the amount of enzyme needed to release acid-soluble fragments equivalent to $\Delta 0.001$ at 280 nm per minute per milligram of protein at pH 7.8 and 37 °C.

The aspartate amino transferase (AST) activity was assayed in liver and muscle tissue homogenates as described by Wooten (1964). The specific activity was expressed as nanomoles of oxaloacetate released per minute per milligram of protein. The procedure adopted for alanine amino transferase (ALT) activity was the same as for AST activity except that the substrate comprised 0.2 M DL-alanine instead of L-aspartic acid. The specific activity was expressed as nanomoles of sodium pyruvate released per minute per milligram of protein. The lactate dehydrogenase (LDH) activity was assayed according to the method of Wroblewski and Laude (1955). The specific enzymatic activity was expressed as units per milligram of protein per minute at 25 °C, where 1 unit was equal to $\Delta 0.01\ OD\ min^{-1}$. The malate dehydrogenase (MDH) activity was assayed in liver and muscle tissue by the method of Ochoa (1955), and the specific enzymatic activity was expressed as unit per milligram of protein per minute at 25 °C, where 1 unit was equal to $\Delta 0.01\ OD\ min^{-1}$.

Oxidative enzyme activity assay

Superoxide dismutase (SOD) activity was assayed according to the method described by Misra and Fridovich (1972), based on the oxidation of epinephrine–adrenochrome transition by the enzyme, and expressed as unit activity (amount of protein required to give 50% inhibition of epinephrine autoxidation). Catalase activity was

assayed according to the method described by Takahara et al. (1960). Enzyme activity was expressed as nanomoles of H_2O_2 decomposed per minute per milligram of protein.

Statistical analysis

All data were statistically analyzed using one-way ANOVA in SPSS package version 16.0, and the significant difference between the means was determined by Duncan's multiple range tests. Comparisons were made at the 5% probability level ($P < 0.05$).

Results

Proximate composition and antinutritional factors

The moisture, crude protein, ether extract, total ash, crude fiber, nitrogen-free extract, and gross energy contents of different experimental diets are shown in Table 1. The proximate composition, amino acid profile, and level of ANFs in DJKM, FJKM, and SBM are given in Table 2. No phorbol esters (PEs) and other ANFs were found in FJKM except tannin (0.13%).

Water quality

Different water quality parameters like temperature (23.2–27.7 °C), pH (7.3–8.3), dissolved oxygen (6.4–7.6 mg L^{-1}), total hardness (223–251 mg L^{-1}), ammonia-N (0.07–0.15 mg L^{-1}), nitrite-N (0.001–0.006 mg L^{-1}), and nitrate-N (0.01–0.05 mg L^{-1}) were well within the optimal range.

Growth performance, nutrient utilization, and survival

The highest WG (%), SGR, and PER values were recorded in the T2 group followed by T3 (Table 3), and there was no significant variation ($P > 0.05$) between T2 and T3. Significantly lower values of these parameters were found in the control group compared to T2 and T3. FCR values showed an opposite trend with a significantly lower value recorded in the T2 group followed by T3. A similar trend vis-a-vis WG (%) was also observed for PER. No mortality was recorded in any of the experimental groups throughout the feeding trial.

HSI, ISI, and digestive enzyme activity

In comparison to the control group, HSI, ISI, and amylase enzyme activity among the fish of various experimental groups did not vary significantly ($P > 0.05$) (Table 4). The protease enzyme activity was found to be significantly ($P < 0.05$) higher in fish of T2 in comparison to fish of the control group, but the enzyme

Table 2 Proximate composition (g kg^{-1} DM) and amino acid profile (g kg^{-1} protein) of untreated *Jatropha* kernel meal, fermented *Jatropha* kernel meal, and soybean meal (Phulia et al. 2017)

Variables (g kg^{-1})	DJKM	FJKM	SBM
Dry matter	921.0	929.5	965.0
Crude protein	578.5	580.0	505.0
Ether extract	6.2	7.3	32.1
Total ash	96.5	98.4	72.3
Crude fiber	91.2	85.0	59.0
Nitrogen-free extract	227.6	229.4	331.6
Indispensable amino acids			
Arginine	19.54	20.30	16.53
Histidine	82.02	84.15	72.60
Isoleucine	19.29	20.50	17.00
Leucine	44.60	46.20	33.90
Lysine	15.62	19.64	18.40
Methionine	5.16	5.80	1.47
Phenylalanine	24.92	25.12	24.84
Threonine	16.34	16.90	15.45
Tryptophan	5.93	6.25	5.92
Valine	25.05	25.90	19.56
Dispensable amino acids (g kg^{-1} protein)			
Alanine	19.53	20.80	12.50
Aspartic acid	47.59	48.61	58.27
Cystine	5.60	6.13	3.27
Glutamic acid	85.62	88.70	93.37
Glycine	27.73	28.94	23.72
Proline	33.03	34.60	6.87
Serine	25.87	27.30	23.56
Tyrosine	10.72	12.60	12.24
Anti-nutritional factors			
Phorbol esters (mg g^{-1})	1.3	N.D.	–
Phytate (% phytic acid equivalent)	8.6	N.D.	2.8
Trypsin inhibitor (TIU mg g^{-1})	21.3	N.D.	N.D.
Tannin (%)	0.38	0.13	0.72

DJKM defatted *Jatropha* kernel meal, FJKM fermented *Jatropha* kernel meal, SBM soybean meal, N.D not detected

Table 3 Growth performance, nutrient utilization, and survival (%) of *Labeo rohita* fingerlings fed different experimental diets

Treatment ^a	Parameters				
	WG (%)	SGR	PER	FCR	Survival (%)
Control	96.37c ± 0.59	1.12c ± 0.01	1.61c ± 0.01	1.87a ± 0.01	100
T1	101.13bc ± 1.31	1.16bc ± 0.01	1.68bc ± 0.02	1.78b ± 0.02	100
T2	107.60a ± 2.14	1.21a ± 0.02	1.79a ± 0.04	1.67c ± 0.03	100
T3	105.04ab ± 1.90	1.19ab ± 0.01	1.75ab ± 0.03	1.71bc ± 0.03	100

Data expressed as mean ± SE; $n = 3$. The mean values in a column bearing different lowercase letters differ significantly ($P < 0.05$)

WG weight gain, SGR specific growth rate, PER protein efficiency ratio, FCR feed conversion ratio

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

activity in fish of T1 and T3 did not differ significantly ($P > 0.05$) with that in fish of the control group (Table 4).

Metabolic enzymes

A similar trend in AST activity was recorded in both muscle and liver of various experimental groups. The AST activity in T2- and T3-fed groups was statistically higher ($P < 0.05$) than that of the control and T1 groups (Table 5). The muscle ALT activity of T2 and T3 was similar but significantly higher ($P < 0.05$) than the control and T1 groups. In the case of liver, the control group exhibited significantly lowest ($P < 0.05$) ALT activity. The LDH activity in muscle did not vary significantly ($P > 0.05$) among the various groups, whereas liver LDH activity was found to be significantly lower in the T1 group but did not differ ($P > 0.05$) to the value recorded in the control and T3 groups. The MDH activity in both the liver and muscle of the fish fed different

experimental diets did not show any significant variation ($P > 0.05$).

Oxidative enzymes

The SOD activity in both the liver and muscle did not show any significant difference ($P > 0.05$) among the various groups (Table 6). A similar observation was recorded in liver catalase enzyme activity ($P > 0.05$) (Table 6).

Whole body composition

The moisture, total ash, total carbohydrate, and gross energy contents did not show any significant difference ($P > 0.05$) among the various groups (Table 7). The lowest crude protein and the highest ether extract values were found in the control group compared to FJKM-fed groups.

Table 4 Hepatosomatic index (HSI), intestinal somatic index (ISI), and digestive enzyme activity of *Labeo rohita* fingerlings fed different experimental diets

Treatment ^a	HSI	ISI	Protease	Amylase
Control	0.86 ± 0.03	2.38 ± 0.06	1.25b ± 0.09	15.38 ± 1.62
T1	0.81 ± 0.04	2.09 ± 0.20	1.32ab ± 0.10	15.10 ± 2.49
T2	0.83 ± 0.02	2.08 ± 0.05	1.53a ± 0.07	18.99 ± 2.15
T3	0.82 ± 0.01	2.06 ± 0.09	1.39ab ± 0.05	16.09 ± 0.33

Mean values in the same column with different lowercase letters differ significantly ($P < 0.05$). Data expressed as mean ± SE, $n = 3$

Unit units per milligram of protein

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

Table 5 Metabolic enzyme activity in the liver and muscle of *Labeo rohita* fingerlings fed different experimental diets

Treatment ^a	AST ^b (M)	AST ^b (L)	ALT ^c (M)	ALT ^c (L)	LDH ^d (M)	LDH ^d (L)	MDH ^e (M)	MDH ^e (L)
Control	16.94b ± 0.78	16.13b ± 0.11	10.15c ± 0.07	15.32c ± 0.37	0.27 ± 0.01	0.16ab ± 0.01	0.51 ± 0.05	0.32 ± 0.05
T1	18.12b ± 0.92	16.74b ± 0.24	10.43c ± 0.03	16.94a ± 0.07	0.25 ± 0.01	0.14b ± 0.01	0.49 ± 0.10	0.28 ± 0.03
T2	20.82a ± 0.47	18.81a ± 0.34	12.34a ± 0.40	17.16a ± 0.04	0.26 ± 0.01	0.17a ± 0.01	0.45 ± 0.03	0.28 ± 0.05
T3	21.29a ± 0.60	18.53a ± 0.05	11.93ab ± 0.17	16.85ab ± 0.14	0.25 ± 0.01	0.16ab ± 0.01	0.45 ± 0.05	0.25 ± 0.05

Mean values bearing different lowercase letters under each column vary significantly ($P < 0.05$). Data expressed as mean ± SE, $n = 3$

M muscle, L liver

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

^b AST, aspartate amino transaminase; specific activity expressed as nanomoles of oxaloacetate released per minute per milligram of protein at 37 °C

^c ALT, alanine amino transaminase; specific activity expressed as nanomoles of sodium pyruvate formed per minute per milligram of protein at 37 °C

^d LDH, lactate dehydrogenase; specific activity expressed as units per minute per milligram of protein at 37 °C, where 1 unit was equal to Δ0.01 OD min⁻¹

^e MDH, malate dehydrogenase; specific activity expressed as units per minute per milligram of protein at 37 °C, where 1 unit was equal to Δ0.01 OD min⁻¹

Discussion

In spite of having a high crude protein content, the presence of toxic/antinutritional factors limits the utilization of plant-based ingredient in fish feed. In order to eliminate or reduce the levels of these deterrent factors and to improve the nutritional value, researchers have adopted several detoxification techniques. In the present study, DJKM was subjected to solid state fermentation (SSF) using *Aspergillus niger*, and the results obtained after analysis indicated that PEs, phytate, and trypsin inhibitors were completely eliminated after fermentation. This is in congruence with Shamna et al. (2015),

who reported no traces of PEs and phytate after fermenting *Jatropha* protein concentrate with *A. niger*. With the elimination of PEs and other ANFs in FJKM, a feeding trial was conducted and various parameters were examined. Water quality parameters were within the optimum range (Debnath et al. 2007) for *L. rohita* fingerlings, which indicates that water quality did not cause any stress to the experimental fish. The groups fed T2 and T3 based diets (200 and 300 g kg⁻¹ FJKM) showed higher WG (%), SGR, and PER, and lower FCR values compared to the control. The improved performance noticed in FJKM-fed groups compared to the SBM group indicates the potential of the former as a

Table 6 Oxidative enzyme activities in liver and muscle of *Labeo rohita* fingerlings fed different experimental diets

Treatment ^a	SOD ^b (M)	SOD ^b (L)	CAT ^c (L)
Control	55.85 ± 0.25	46.98 ± 0.57	2.61 ± 0.09
T1	55.94 ± 0.23	44.98 ± 1.42	2.86 ± 0.15
T2	56.79 ± 0.32	46.90 ± 0.47	2.68 ± 0.05
T3	55.90 ± 0.32	44.95 ± 0.92	2.44 ± 0.11

Data expressed as mean ± SE, $n = 3$

M muscle, L liver

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

^b SOD, superoxide dismutase; specific activity expressed as unit activity (amount of protein required to give 50% inhibition of epinephrine auto oxidation) per minute per milligram of protein

^c CAT: catalase; specific activity expressed as nanomoles H₂O₂ decomposed per minute per milligram of protein

Table 7 Whole body composition (on wet weight basis) of *Labeo rohita* fingerlings fed different experimental diets

Parameters	Treatments ^a			
	Control	T1	T2	T3
Moisture (%)	75.34 ± 0.13	74.96 ± 0.23	74.79 ± 0.35	74.58 ± 0.15
CP (%)	15.64b ± 0.20	16.20a ± 0.09	16.33a ± 0.04	16.34a ± 0.08
EE (%)	4.23a ± 0.14	3.82b ± 0.13	3.80b ± 0.04	3.84b ± 0.11
TA (%)	3.25 ± 0.13	3.17 ± 0.11	3.20 ± 0.15	3.22 ± 0.05
TC (%)	1.54 ± 0.32	1.85 ± 0.39	1.88 ± 0.48	2.02 ± 0.34
GE (MJ kg ⁻¹)	4.73 ± 0.04	4.73 ± 0.08	4.75 ± 0.14	4.79 ± 0.12

Mean values in the same row with different lowercase letters differ significantly ($P < 0.05$). Data expressed as mean ± SE, $n = 3$. Gross energy (MJ kg⁻¹) = $[19 \times \text{CP (g kg}^{-1}\text{)} + 36 \times \text{EE (g kg}^{-1}\text{)} + 15 \times \text{TC (g kg}^{-1}\text{)}]$ kJ kg⁻¹ 1000⁻¹ (Smith 1981)

CP crude protein, EE ether extract, TA total ash, TC total carbohydrate, GE gross energy

^a Control, 0 g kg⁻¹ FJKM; T1, 100 g kg⁻¹ FJKM; T2, 200 g kg⁻¹ FJKM; T3, 300 g kg⁻¹ FJKM in replacement of 0, 33.33, 66.67, and 100% SBM, respectively

dietary protein source and can be used to replace SBM completely (100%) in the diet of rohu without any detrimental effects on growth performance and nutrient utilization. A similar observation was also made by Shamna et al. (2015) in *L. rohita* fingerlings fed fermented *Jatropha* protein concentrate (FJPC). Hassaan et al. (2016) reported 50% replacement of fish meal by fermented *J. curcas* seed meal in the diet of Nile tilapia without compromising fish performance. Latif et al. (2015) also observed that common carp fed 50% detoxified *Jatropha* protein isolate performed similar to those fed fish meal-based control diet. Further, it was observed that feeding FJKM caused no mortality among the groups, and this can be attributed to the complete removal of phorbol esters which causes death in fish (Becker and Makkar 1998; Shamna et al. 2015). Also, the HSI and ISI values did not differ significantly among the fish of different experimental groups, which is in agreement with the observation of Shamna et al. (2015), who reported that *L. rohita* fingerlings fed SPC and fermented JPC showed no significant differences in the HSI and ISI values. This indicates that feeding FJKM to rohu in the present study did not alter the physiological functions and survival of the fish.

The improvement in the performance of an animal in terms of growth and nutrient utilization is a close reflection of the digestive enzyme influence on the digestion processes (Lemieux et al. 1999; Fawole et al. 2016a, b). In the present study, the activity of protease enzyme was found to be higher in FJKM-fed groups compared to the control. This indicates better protein utilization (PER) among the fish fed FJKM especially fish of T2 to reflect improved growth performance (%WG and SGR) which might be

due to complete elimination of the inherent toxic factors in FJKM, since no inhibitory effects were noticed on the protein-digesting enzyme and organ integrity of the fish as reported in our earlier study (Phulia et al. 2017). In congruence with our findings, Shamna et al. (2015) reported that *Labeo rohita* fingerlings fed fermented *Jatropha* protein concentrate (JPC) recorded higher protease enzyme activity compared to those fed non-fermented JPC, which contains high level of phorbol esters, trypsin inhibitors, and phytate. The insignificant variation in the amylase activity among the various groups in the present study is in line with the observation of Kumar et al. (2012) in common carp fed detoxified *Jatropha* protein isolate.

The activities of transaminase enzymes (AST/GOT and ALT/GPT) exhibit both synthesis and degradation effects on amino acids. AST and ALT are involved in the transfer of amino nitrogen from amino acids to keto acids to form new amino acids, which take part in the synthesis of body protein. In the present investigation, increased AST and ALT activities in the liver and muscle of fish fed 200 and 300 g kg⁻¹ FJKM compared to fish fed SBM-based control diet and 100 g kg⁻¹ FJKM indicate optimum metabolism of nutrients, thus leading to increased growth as seen in the present study. In congruence to the present finding, Shamna et al. (2015) reported higher AST and ALT activities at tissue level in fish fed FJPC-based diet compared to those fed untreated JPC. The authors attributed the lower activities in JPC groups to the liver damage caused by the action of toxic components (PEs) present in the diet, thus resulting in the leakage of the enzyme into the blood stream (Shamna et al. 2015). However, the higher

activities of both transaminase enzymes in the present study are indicative of no traces of PEs in the diet and normal liver function.

LDH enzyme is known to be active when there is an oxygen debt in the tissue, causing pyruvate to be converted to lactate by LDH, and NADH is converted to NAD⁺ to maintain redox potential in the tissue (Murray et al. 2000; Shamna et al. 2015). The similar hepatic LDH activity among the fish fed 0, 200, and 300 g kg⁻¹ FJKM diets suggests that there was no oxygen shortage in the tissue and that anaerobic metabolism was not active. On the contrary, Shamna et al. (2015) observed increased LDH activity in *L. rohita* fingerlings fed diet containing phorbol esters (untreated JPC), which invariably resulted in an increased MDH enzyme activity. Increased MDH activity indicates increased energy demand in fish (Shamna et al. 2015) especially when under stress, but in the present study, MDH activity in both the muscle and liver of different experimental groups was found to be similar ($P > 0.05$). In support of our findings, Shamna et al. (2015) reported that fish fed control and FJPC-based diets showed lesser activity of these enzymes in comparison to those fed diet containing non-fermented JPC.

SOD and catalase are important antioxidant enzymes that play a protective function against damaging effects of free radicals in the organism (Shamna et al. 2017). They are reported to act as a biomarker for stress and immunity in fish (Sagstad et al. 2007; Fawole et al. 2016a, b). In the current study, inclusion of FJKM in the diets of *L. rohita* did not cause any stress to the fish as indicated by similar SOD and catalase activities in fish of control and FJKM-fed groups.

The present observation of increasing protein and decreasing body lipid content in fish of FJKM groups in comparison to those fed control might be due to better protein utilization in the fish of former groups leading to more protein accretion in the fish muscle attributing to higher growth. This finding proves that FJKM is a good dietary protein source in replacement of SBM for carp diet. Our findings corroborated the observation of several researchers (Kumar et al. 2011; Nagel et al. 2012; Slawski et al. 2013; Saha and Ghosh 2013; Shamna et al. 2015; Hassaan et al. 2016).

Conclusion

Fermentation of *Jatropha* kernel meal completely removes the highly toxic factor phorbol esters, which

showed growth performance of Indian major carp *L. rohita* at par with SBM; however, further evaluation of this high protein ingredient in advanced juveniles and adult stage are warranted for long-term feeding trials.

Acknowledgements We gratefully acknowledge the Director and Vice Chancellor, ICAR-Central Institute of Fisheries Education, Mumbai, for providing the facilities to conduct this research work. The first author duly acknowledges the UGC, New Delhi, for awarding Rajiv Gandhi National Fellowship to pursue the Ph.D program.

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