



Haemato-immunological and physiological responses of *Labeo rohita* fingerlings to dietary fermented *Jatropha curcas* protein concentrate

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ABSTRACT

Jatropha protein concentrate (JPC) prepared from *Jatropha* seed cake is toxic due to the presence of phorbol ester and antinutritional factors like tannin, trypsin inhibitor, phytate and non-starch polysaccharides. JPC was detoxified by solid state fermentation (SSF) and a feeding trial of 45 days was conducted to study the response of feeding fermented JPC (FJPC) on growth, haemato-immunological and physiological responses in rohu fingerlings. Seven iso-nitrogenous diets such as control (without JPC or FJPC), J5 (5% JPC), J10 (10% JPC), J20 (20% JPC), FJ5 (5% FJPC), FJ10 (10% FJPC) and FJ20 (20% FJPC) were prepared and fed twice daily. The weight gain and specific growth rate values showed an overall, linear and quadratic trend with similar value recorded in the control and FJ fed groups. Feed efficiency also showed an overall significant effect with a higher feed efficiency value recorded in the control (60.50) group which was similar to FJ (54.21–58.37) fed groups, while JPC fed groups registered the lowest value. The haematological studies showed a significantly ($p < 0.05$) lower red blood cells (RBC) and haemoglobin in all JPC fed groups and 5% FJPC group compared to control and other FJPC groups. The 10% and 20% JPC fed groups showed the highest blood glucose level than any other groups. Serum total protein and albumin followed similar trend as that of RBC and haemoglobin. The highest globulin value were observed in FJ10 group which was significantly different ($P < 0.05$) to other groups and lowest value was recorded in J20 group. The superoxide dismutase (SOD) activity in liver was higher in J20, while in muscle, J10 and J20 registered the highest value compared to other groups ($p < 0.05$). Our study revealed that protein concentrate prepared from *Jatropha* cake cannot be fed directly to rohu without detoxification and solid state fermentation appears to be an ideal approach. Overall, FJPC can be utilized in the diet of rohu at 20% without any detrimental effect on haemato-immunological and physiological response.

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1. Introduction

Several alternative plant protein source ingredients have been studied over the last few decades due to the reduction in fish meal production and increasing cost of fish meal in the industry (Jackson, 2006; Hardy, 2010). Soybean meal (SBM) is one of the most studied plant protein source and are considered as a good ingredient for aquafeed production (Kaushik et al., 1995). However, competition with human food and high demand in other feed industry has led to a hike in the price of soybean meal (FAO, 2006). Also, Ray et al. (2013) reported that the yield trends of most of the crops like rice, soybean, groundnut etc., are insufficient to meet the human requirement in the coming years. Hence, it is imperative to explore an alternative, less expensive, non-edible ingredient to satisfy the aquaculture requirement. One of the best alternatives in this regard is to utilize the industrial wastes and by-products which are cheap, surplus and eco-friendly. Global oil industry is now attempting to utilize non-edible seeds like rubber seeds, neem seeds, pongamia, karanja, mahua, *Jatropha* etc., for biodiesel production as there is debate in utilizing edible seeds for the same (Butler, 2006; Meher et al., 2006). A promising candidate of this industry is *Jatropha curcas* (family Euphorbaceae), a drought resistant tropical shrub. Large-scale *Jatropha* cultivation projects of about 12.8 million hectares yielding 2 t/ha of oil by 2015 has been initiated worldwide (Global Exchange for Social Investment market study GEXSI, 2008). The main by-product of bio-diesel industry utilizing *jatropha* is *Jatropha* Seed Cake (JSC) and its protein content ranges from 27 to 33% (Makkar and Becker, 2008). As JSC contains less protein, the better way to enhance its utilization is to convert them into a protein concentrate in line with soybean protein concentrate. Protein concentrate contain high protein and low fibre content. There are several method for the preparation of protein concentrate like iso-electric precipitation (Devappa and Swamylingappa, 2008), alkaline extraction (Tobin and Carpenter, 1978), sedimentation-flotation (Parrado et al., 1991), and ultra-filtration method (McClements, 2013). Different research works were conducted recently to study the inclusion level of various protein concentrate in fish like canola protein concentrate in atlantic salmon and rainbow trout (Burr et al., 2013; Slawski et al., 2013), Pea protein concentrate in juvenile crayfish (Fuertes et al., 2014), and soybean protein concentrate in African catfish and *Tinca tinca* (Rodríguez et al., 2014). With this backdrop, we prepared JPC using iso-electric precipitation method. Even though the extraction itself reduced the ANFs, the presences of phorbol ester still remain high above the tolerance level (Shamna et al., 2015). In the present study we detoxified JPC using solid state fermentation (SSF) and studied the effect of JPC and FJPC on the growth indices, hemato-immunological parameters and stress enzyme activities in *Labeo rohita* fingerlings.

2. Materials and methods

2.1. Preparation and detoxification of *jatropha* protein concentrate

Jatropha protein concentrate (JPC) was prepared as described in the complementary work by Shamna et al. (2015). JPC was detoxified by solid state fermentation, in summary, *Aspergillus niger* (MTCC 281) obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, was subculture in Czapek Yeast Extract Agar (CYA) and the fungal spores were collected in Tween-80 solution and diluted to get 10^7 – 10^8 spores mL⁻¹ by serial dilution. Fermentation was conducted for a period of seven days on dried JPC at 30° C aerobically.

2.2. Diet formulation

Seven iso-nitrogenous (about 340 g/kg crude protein) experimental diets were formulated (Table 1). Control diet contained soy protein concentrate (SPC) as major protein source and devoid of JPC and FJPC, whereas the treatment groups contained 50, 100 and 200 g/kg JPC or FJPC, replacing equal amount of SPC in the diet. Other ingredients like fishmeal, groundnut oil cake, rice flour, wheat flour, corn flour were identical for all the diets. Oil and vitamin-mineral mixture were included in all the diets at equal level. Carboxymethyl cellulose (CMC) was used as binder and butylated hydroxyl toluene (BHT) was used as antioxidants in the diets.

2.3. Experimental set-up and sampling

The experiment was conducted in 21 plastic rectangular tubs (80 × 57 × 42 cm, 150 L capacity) covered with perforated lids previously treated and cleaned with potassium permanganate solution (5 ppm). Two hundred and ten fingerlings (initial weight 6.35 ± 0.02 g) were randomly distributed in seven distinct experimental groups like Control; J5 (50 g/kg JPC); J10 (100 g/kg JPC); J20 (200 g/kg JPC); FJ5 (50 g/kg FJPC); FJ10 (100 g/kg FJPC) and FJ20 (200 g/kg FJPC) in triplicates. The experiment was conducted for a period of 45 days and feeding was done to satiation level. Fishes were starved overnight before taking the body weight at every 15 days. Mortality was recorded throughout the experiment. After 45 days of feeding, six fishes were sampled from each tank with a total of 18 from each treatment and anesthetized using CIFEALM (CIFE, Mumbai, India) at 200 µl/L. Tissues of different organs (liver and muscle) were dissected out and homogenized with 0.25 M chilled sucrose solution in a glass tube using teflon coated mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 min in a cooling centrifuge (REMICPR-24, India). The

Table 1

Feed formulae and proximate composition of feed.

Ingredients	Diet code						
	Control	J5	J10	J20	FJ5	FJ10	FJ20
Fish meal ^a	50	50	50	50	50	50	50
Ground nut oil cake ^a	70	70	70	70	70	70	70
Soya protein concentrate ^a	250	200	150	50	200	150	0
Jatropha protein concentrate ^a	0	50	100	200	0	0	0
Fermented Jatropha protein concentrate	0	0	0	0	50	100	200
Rice flour ^a	200	200	200	200	200	200	200
Wheat flour ^a	180	180	180	180	180	180	180
Corn flour ^a	153	153	153	153	153	153	153
Sunflower ^b ; cod liver oil ^c (2:1)	60	60	60	60	60	60	60
Vit.Min.Mix ^d	20	20	20	20	20	20	20
Carboxy methyl cellulose (Himedia India)	15	15	15	15	15	15	15
BHT(Himedia, India)	2	2	2	2	2	2	2
Proximate composition (g kg ⁻¹ DM)							
Dry matter	936.9 ± 1.6	932.7 ± 0.4	933.1 ± 4.0	936.7 ± 0.8	937.4 ± 0.1	936.3 ± 0.3	953.4 ± 1.4
Crude protein	338.1 ± 2.6	340.3 ± 4.1	339.1 ± 5.2	338.3 ± 2.6	345.2 ± 4.0	339.1 ± 2.3	337.3 ± 2.1
Ether Extract	68.2 ± 2.0	75.6 ± 0.5	75.8 ± 2.3	72.5 ± 4.1	72.9 ± 6.0	74.2 ± 4.2	74.0 ± 2.4
Total Ash	59.0 ± 5.3	52.2 ± 2.6	76.3 ± 3.8	65.6 ± 2.2	73.4 ± 1.2	85.0 ± 0.4	81.6 ± 0.5
Crude Fibre	82.9 ± 1.2	85.5 ± 1.3	84.7 ± 0.6	84.2 ± 1.3	85.9 ± 1.7	84.0 ± 0.8	87.1 ± 1.0
Nitrogen free extract	451.7 ± 11.0	446.1 ± 4.5	432.1 ± 8.4	439.2 ± 5.0	422.4 ± 4.1	427.5 ± 6.3	425.9 ± 3.3
Calculated energy* (MJ kg ⁻¹)	17.17 ± 0.6	17.43 ± 0.09	17.18 ± 0.06	17.14 ± 0.12	17.03 ± 0.14	17.02 ± 0.09	17.06 ± 0.06

J- Jatropha protein concentrate; FJ – Fermented Jatropha protein concentrate.

Vitamin A, 5,500,000 IU; Vitamin D3, 1,100,000 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.

Calculated energy* (MJ kg⁻¹) = [16.74 × CP (g kg⁻¹) + 37.7 EE (g kg⁻¹) + 16.74 × TC (g kg⁻¹)]/1000.^a Purchased from local dealers, Mumbai, India.^b Marico Industries Limited, Mumbai, India.^c Universal Medicare Private Limited, Mumbai, India.^d Composition of vitamin mineral mix (PREEMIX PLUS, Himedia, India) (quantity/2.5 kg).**Table 2**Growth performance of *Labeo rohita* fingerlings fed different experimental diets.

Variables	Treatments							SEM ^a	p-values		
	Control	J5	J10	J20	FJ5	FJ10	FJ20		Overall	Linear	Quadratic
Initial weight (g)	6.27	6.43	6.31	6.41	6.33	6.32	6.35	0.055	0.403	0.830	0.398
Final weight (g)	12.79	11.94	9.48	9.35	12.36	12.55	12.88	0.234	<0.001	0.003	<0.001
Weight gain (g)	6.52	5.51	3.17	2.95	6.04	6.24	6.53	0.217	<0.001	0.002	<0.001
FE	60.50	51.96	33.52	32.22	54.21	57.82	58.37	2.039	<0.001	0.030	<0.001
SGR	1.45	1.36	1.14	1.12	1.41	1.42	1.45	0.019	<0.001	0.002	<0.001

Weight gain = Final weight (g) – Initial weight (g); Feed efficiency (FE) = (final weight – Initial weight) × 100/dry feed given; Specific growth rate (SGR, g/day) = 100 × (ln final body weight – ln initial body weight)/experimental duration in days. Estimated Marginal Means.

^a Standard error of the mean.

supernatant was collected and stored at –20 °C until further use. A 5% homogenate was prepared for the tissues of interest for enzymes assay.

2.4. Growth parameter and analysis of proximate composition

Growth parameters such as weight gain, specific growth rate (SGR) and feed efficiency (FE) were calculated using standard formula as given beneath Table 2. Proximate analysis of feed and tissue were performed as per the standard method of AOAC (1995) in the Nutrition Laboratory of FNBP division of CIFE. Samples were analyzed for dry matter, crude protein, lipid, ash, and fibre.

2.5. Enzyme analysis

2.5.1. Superoxide dismutase (SOD) and catalase (CAT)

Superoxide dismutase 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 auto oxidation). Catalase was assayed according to the method described by Takahara et al. (1960). Enzyme activity was expressed as nano moles H₂O₂ decomposed/min/mg protein.

2.6. Analysis of haemato- immunological parameters

2.6.1. Collection of blood and serum

Each fish was anesthetized using CIFECALM (CIFE, Mumbai, India) at 200 µl/L before taking blood. Blood was drawn from caudal vein (*Vena caudalis*) using a tuberculin syringe, which was previously rinsed with 2.7% EDTA solution. Collected blood was then transferred immediately to test tube containing thin layer of EDTA powder and shaken gently in order to prevent haemolysis of blood. Using this blood, we determined the haemoglobin content, red blood cells (RBC), white blood cells (WBC) and nitroblue tetrazolium (NBT) assay. For serum, blood was collected without anticoagulant from six fishes for each treatment. For getting the clot, eppendorf tube containing blood was kept in tilted position at room temperature for an hour. After centrifugation at 3000 x g for 5 min the sera were kept in –20 °C until analysis of immunological and biochemical parameters

2.6.2. Blood parameters

The haemoglobin content of blood was analyzed following the cyanmethemoglobin method (Van Kampen and Zijlstra, 1961) using Drabkins Fluid (Qualigens, GlaxoSmithkline Pharmaceutical Ltd, India). Blood (20 µl) was mixed with 5 ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at wavelength of 540 nm. Total erythrocyte count was done using haemocytometer as described by Hendrick (1952), and following the method of Shaw (1930), total leucocyte count was determined. Serum glucose was estimated using a diagnostic kit (GOD/POD; Innoline (TM) Merck Specialities, Mumbai, India).

2.6.3. Nitrobluetetrazolium (NBT) assay and lysozyme activity

Nitroblue tetrazolium assay was done by the method of Secombes (1990) as modified by Stasiak and Baumann (1996). Fifty microliter of blood was placed into the wells of a flat bottom micro titre plate and incubated at 37 °C for 1 h to allow adhesion of cells. The supernatant was discarded and the wells were washed three times with phosphate buffer saline. After washing, 50 µl of 0.2% NBT were added and incubated for 1 h. The cells were then fixed with 100% methanol for 2–3 min and washed three times with 70% methanol. The plates were air-dried and 60 µl of 2 N potassium hydroxide and 70 µl dimethyl sulphoxide were added to each well. The OD of the turquoise blue coloured solution was measured in micro plate reader (BioTek Power Wave 340, India) at 620 nm.

Lysozyme activity was studied by the method of Anderson and Siwicki (1995). Into a cuvette containing 3 ml of *Micrococcus luteus* (MTCC 106) suspension in phosphate buffer ($A_{450} = 0.5$), 50 µl of diluted serum sample was added. After proper mixing for 15 s, reading was taken in spectrophotometer at 450 nm at regular intervals of 15, 30 and 270 s. A unit of lysozyme activity was defined as the amount of sample causing a reduction in absorbance of 0.001/min and lysozyme activity was expressed as U/min.

2.6.4. Serum total protein, albumin and globulin

Serum protein was estimated using a diagnostic kit (Total protein and albumin kit, Innoline^(TM) Merck Specialities Pvt, Ltd, India). Albumin was estimated by the bromocresol green binding method (Doumas et al., 1971) and the absorbance was measured at 630 nm. Albumin was subtracted from serum total protein to obtain globulin values.

2.7. Statistical analysis

Data were statistically analyzed using General Linear Model procedure of IBM SPSS package (V.20), and means compared by contrast analysis. The overall treatments effects were determined and polynomial contrasts were used to test both the linear and quadratic effects of dietary inclusion. All data were compared at 5% probability level.

3. Results

3.1. Growth performance

The growth performance indices like final weight, weight gain (WG), feed efficiency (FE) and specific growth rate (SGR) of *Labeo rohita* fingerlings fed different diets are shown in Table 2. The growth performance indices showed an overall significant effect ($p < 0.001$), and followed both linear and quadratic trend ($p < 0.01$). In all the growth performance indices, FJ20 fed group and control recorded the highest value, while J10 and J20 registered the lowest value.

3.2. Whole fish body composition

Estimated whole body compositions (on wet weight basis) of all the experimental fish are given in Table 3. There is an overall significant effects in whole body fish composition and the variance was found to follow both the linear and quadratic trends ($P < 0.01$). A significantly higher dry matter was recorded in FJ20 group. The lowest protein content was observed in J20, while control recorded the highest value. Ash content showed an increasing linear and quadratic trends ($P < 0.001$).

Table 3Proximate composition of the whole body composition of *Labeo rohita* fingerlings of different experimental groups (% as fed basis).

Treatments	Dry matter	Crude protein	Ether extract	Ash
Control	24.88	16.78	4.12	2.74
J5	24.54	16.32	4.07	2.95
J10	24.23	15.84	3.70	3.01
J20	23.66	15.48	3.69	3.09
FJ5	24.90	16.24	4.48	3.20
FJ10	24.91	16.28	4.51	3.28
FJ20	25.17	16.40	4.31	3.11
SEM ^b	0.138	0.028	0.045	0.024
Overall	<0.001	<0.001	<0.001	<0.001
Linear	0.006	<0.001	0.002	<0.001
Quadratic	<0.001	<0.001	<0.001	<0.001

Estimated Marginal Means. ^bStandard error of the mean.**Table 4**Haemato-immunological parameters and glucose level in *Labeo rohita* fingerlings fed with different diets.

Variables	Treatments							SEM ^b	p-values		
	Control	J5	J10	J20	FJ5	FJ10	FJ20		Overall	Linear	Quadratic
RBC	1.65	0.94	1.12	1.19	1.18	1.50	1.60	0.016	<0.001	<0.001	<0.001
Haemoglobin	5.72	3.70	3.61	3.87	3.77	5.17	5.18	0.035	<0.001	<0.001	<0.001
WBC	1.34	1.54	1.40	1.65	1.57	1.50	1.42	0.007	<0.001	<0.001	<0.001
Total protein	5.26	4.65	3.69	3.25	4.48	4.45	4.54	0.151	<0.001	0.038	<0.001
Albumin	2.21	1.79	1.27	1.17	1.67	1.38	1.60	0.050	<0.001	<0.001	<0.001
Globulin	3.04	2.86	2.42	2.07	2.81	3.07	2.94	0.082	<0.001	0.283	<0.001
Lysozyme	170.49	203.28	170.49	163.93	144.26	183.60	157.38	0.090	<0.001	<0.001	<0.001
NBT	0.34	0.29	0.28	0.25	0.33	0.33	0.31	0.026	0.199	0.700	0.082
Glucose	48.33	50.01	62.80	71.93	50.92	52.10	55.35	1.349	<0.001	0.083	<0.001

Estimated Marginal Means. ^bStandard error of the mean. Unit of glucose (mg/100 ml), RBC- Red blood cells (10^6 cells/mm³), WBC- White blood cells (10^3 cells/mm³), lysozyme activity (U/min), albumin (g/dl), globulin (g/dl) and total protein (g/dl), NBT-Nitroblue tetrazolium.

3.3. Haemato- immunological parameters

3.3.1. Haematological parameters

Haematological parameters of the different experimental groups are given in Table 4. The RBC counts linearly and quadratically decreased across the treatments up to FJ5 groups, however, the value increased afterwards in FJ10 and FJ20 fed groups. Haemoglobin also follows the same trend like RBC with significantly lowest value recorded in J5, J10, J20 and FJ5 fed groups ($p < 0.05$). WBC showed overall significant effect, with the lowest value recorded in control ($p < 0.05$).

3.3.2. Total protein, albumin, globulin and lysozyme activity in serum

Serum total protein, albumin, globulin and lysozyme activity of different experimental groups are given in Table 4. There was a significant difference in serum protein level among various experimental groups. The control group exhibited highest value followed by J5, FJ5, FJ10, and FJ20 groups. Inclusion of JPC and FJPC showed overall significant reduction in serum albumin level and the reduction followed both linear and quadratic trends. Lowest value of the serum albumin level was observed in the J20 group. There was an overall significant effect in globulin level but showed no linear trend. The lysozyme activity showed both linear and quadratic effects, with the highest value recorded in J5 group ($P < 0.05$).

3.3.3. Nitroblue tetrazolium (NBT) assay and blood glucose level

The superoxide anion production measured by NBT reduction was not affected by the dietary inclusion of JPC and FJPC ($P > 0.05$). The NBT reduction value showed no linear and quadratic trends. The blood glucose level showed overall significant effects but no linear trend was observed. The highest glucose level was recorded in J20 fed fish, whereas the control group registered the lowest value.

3.4. Oxidative enzymes activity

SOD activities of both liver and muscle can be seen in Table 5. The SOD activity in the liver showed both linear and quadratic effects, with the highest value recorded in J20 group ($P < 0.05$). However, SOD activity in the muscle showed no defined trend ($P > 0.05$), but recorded an overall significant effects. Catalase enzyme activity did not show overall and linear effects, but quadratically reduced ($P < 0.05$) and the lowest value was found in FJ20.

Table 5Antioxidant enzymes activity in liver (L) and muscle (M) tissues of *Labeo rohita* fingerlings fed different experimental diets.

Variables	Treatments							SEM	p-values		
	Control	J5	J10	J20	FJ5	FJ10	FJ20		Overall	Linear	Quadratic
SOD (Liver)	31.38	32.20	34.72	37.16	29.30	29.43	30.54	0.576	<0.001	<0.001	<0.001
SOD (Muscle)	29.81	31.54	32.81	32.92	27.85	31.04	32.11	0.851	0.002	0.842	0.619
Catalase (Liver)	0.06	0.05	0.06	0.07	0.05	0.05	0.04	0.06	0.060	0.104	0.016

Estimated Marginal Means. ^bStandard error of the mean. Catalase specific activity is expressed in nanomoles H₂O₂ decomposed/min/mg of protein. SOD activity expressed in micromoles/mg protein/min.

4. Discussion

Jatropha seed cake contains several antinutritional factors like phytate, trypsin inhibitor, tannin and non-starch polysaccharides (Makkar et al., 1998; Kumar et al., 2011). However, the protein content (27–33%) in the seed cake was promising. So in our study, to remove the antinutritional factors and enhance the nutrient utilization potential of Jatropha seed cake, the protein was extracted from the seed cake using the principle of isoelectric precipitation (Kumar et al., 2011). Although, reduction in anti-nutritional factors was apparent after protein extraction but the phorbol ester (1.4 g kg⁻¹; Shamna et al., 2015) remain bounded to the protein, and its concentration in the concentrate is relatively high. Hence, JPC was subjected to solid state fermentation (SSF). We observed in our previous study that up to 5% inclusion of JPC and 20% inclusion of FJPC did not cause any adverse effect on growth and nutrient utilization in rohu fingerlings (Shamna et al., 2015). In the present study, we examined the haemato-immunological status of the fish in response to JPC and FJPC with a soybean protein concentrate as control.

4.1. Growth performance and whole body composition

The high growth rate recorded in the control and fermented Jatropha (FJ) concentrate fed groups indicates the potential of the later for usage as a plant protein source ingredient in the diets for *L. rohita* fingerlings. As reported in the first part of this study, high concentration of phorbol ester, trypsin inhibitor and tannin observed in the JPC resulted in a decreased activity of digestive enzyme (Shamna et al., 2015), and this could be the reason for the growth depression recorded in J5 and J10 fed groups. Anti nutritional factors (ANFs) such as trypsin inhibitor and phytate have been reported to inhibit the activity of protein digesting enzymes and forms complexes with minerals, thereby causes reduction in growth, nutrient utilization and feed efficiency as seen in the group fed jatropha protein concentrate diets (Krogdahl et al., 1994; Francis and Becker, 2001; Kumar et al., 2010). These result is complementary to our earlier report (Shamna et al., 2015) and in accord with Sharma et al. (2014) who reported that feeding rubber kernel meal containing a high concentration of ANFs to *L. rohita* fingerlings resulted in reduced weight gain and SGR value.

The decrease in the crude protein and lipid contents observed in fish fed J10 and J 20 diets could be attributed to the ANFs role in inhibiting the activities of intestinal enzymes (Francis and Becker, 2001; Kumar et al., 2010), thereby limiting the utilization of nutrients. Conversely, fermentation technology successfully reverses this effect as the fish fed FJ-based diets performed similar to the control group. Hence, similar lipid contents value recorded in FJ fed groups and control could be due to the ability of plant protein to increase the hepatic lipogenic enzyme activities in fish (Skonberg et al., 1997; Kaushik et al., 2004; Akinleye et al., 2010; Kumar et al., 2011). The whole body ash content increased linearly as the inclusion level of JPC and FJPC increases, indicating sufficient dietary mineral supply and its absorption. Similar results were reported by other researchers (Kumar et al., 2011; Nagel et al., 2012; Slawski et al., 2013).

4.2. Haemato-immunological status of the fish

Haematological parameters are considered to be general indicators of fish health (NRC, 1993). In the present study, a significantly higher RBC content was recorded in the control group followed by FJ20 fed group. Similar pattern was also observed for Hb levels. From our earlier study, it was clear that the tannin, phytate and trypsin inhibitor contents in the JPC groups were higher (17 g/kg, 26.5 g/kg, 2.18 TIU g/kg, respectively) than the FJPC groups (11 g/kg, not detected, 1.28 TIUg/kg) (Shamna et al., 2015), and this could be the reason for decreased RBC and Hb values recorded in JPC fed groups. It is well known that tannin can affect the absorption of iron and cyanocobalamine, and could cause low haemoglobin formation and fragmentation of erythrocytes (Francis and Becker, 2001). Reduced protein metabolism due to high level of trypsin inhibitor (TI) and phytate could also led to a reduction in the RBC synthesis in the body, thereby resulting in reduced haemoglobin content (Akinleye et al., 2010). Hence, it can be said that inclusion of JPC without detoxification at higher level is detrimental to fish health due to the presence of toxic components, but could be improved by fermentation as seen in the present study. The WBC showed an overall significance effects with higher value observed in J20 group.

WBC plays a significant role in immune defense response against deleterious effect of pathogen and defend the body against invasion by foreign substances, including toxin (Roberts, 1978; Ajima et al., 2015). Thus, the higher leucocyte counts recorded in J20 group suggest immune response strategy by the fish to combat foreign body.

The similar respiratory burst activity of the control and FJ groups, suggest that there is no adverse effects on adding FJPC in the diets for rohu. Similar to our work, Wang et al. (2011) reported that detoxified *Jatropha* kernel meal with no traces of PE could replace 50% soybean meal in growing pig diet. Also a report by Kumar et al. (2010) stated that *Jatropha* kernel meal subjected to 60 min detoxification process could replace 50% of fishmeal in the diet of *Cyprinus carpio*. But, according to Li et al. (2015) partially detoxified (PE –8.25 mg/kg) can replace 45% of soybean meal protein in growing pig diet without altering the heamato- immunological status. Nevertheless, feeding non-treated *jatropha* protein concentrate (JPC) as tested in the present study resulted in a decreased respiratory burst activity, and this could be due to some toxic factors (e.g. phorbol ester, phytate, trypsin inhibitor) present in the JPC based diets. It has been reported that some deterrent factors when present at higher level could cause immunosuppression of innate immunity in fish (Deng et al., 2015).

Another important parameter showing the nutritional status and health of the fish are the total protein concentration of serum (Martinez, 1976) and these circulating mobile proteins include albumin and globulin, which plays a major role in the immune response of fish (Kumar et al., 2010). The control group showed highest value of total protein followed by all levels of FJPC and 5% JPC groups. The lower values of protein, albumin and globulin observed in 10 and 20% JPC fed groups, may be due to the detrimental effects of phorbol ester and other anti-nutritional factors, thus, resulting in an impaired protein metabolism and suppressed immunity. The anti-nutrients level published in our previous study (Shamna et al., 2015) is supporting this assertion. In line with our result, Xu et al. (2012) also reported a low serum protein levels when fishmeal was replaced with soybean protein isolate in the diet of *Acipensers chrenckii*. Lysozyme act as a defensive mechanism against external agents and is present mainly in mucus and serum (Ellis, 1990). Increased level of lysozyme is an indicator of better immunity. Some studies have interpreted the increase in non- specific immune parameters (Rumsey et al., 1994; Krogdahl et al., 2000) in plant protein fed fish as due to their immunostimulating potential resulting from the presence of ANFs, and this may be the reason for higher values recorded in J5, J10 and J20. Similar observation was made by Kumar et al. (2010)

4.3. Blood glucose and oxidative enzyme activity

High blood glucose level in fish has been considered as secondary level stress indicator (Hattingh, 1976) and also indicates high energy demanding condition (Manush et al., 2005). It is reported that during all types of stress catecholamine secretion increases, which in turn elevate the glucose level in blood for energy production to mitigate the stressful condition (Nakano and Tomlinson, 1967). In the present study, blood glucose level was found to be highest in fish fed with 20% JPC compare to all other groups. This may be due to phorbol ester-induced stress which prevailed in the fishes of these treatments (J10–J20) groups. Akinleye et al. (2010) also reported a higher blood glucose level in Nile tilapia fed *Jatropha platyphylla* kernel meal based diet. SOD and catalase are important anti-oxidant enzymes that play a protective function against damaging effects of free radicals in organism and are reported to act as a biomarker for oxidative stress and immunity in fish (Sagstad et al., 2007). In the present study, the significantly higher SOD activity in the liver of J20 fed group may be due to the effect of toxic factors present in the JPC diets which could trigger oxidative stress. There are several reports saying that on inclusion of plant ingredients containing antinutritional factors in animal diet causes an increased oxidative stress (Olsvik et al., 2011; Sitjà-Bobadilla et al., 2005). Similar to our observation, replacement of soybean meal with rubber seed meal in tilapia has also been reported to trigger oxidative stress due to the presence of cyanide (Deng et al., 2015). Zeng et al. (2012) also reported that on higher inclusion level of cotton seed meal in grass carp diet causes elevated activity in SOD and catalase due to the toxic component in it.

5. Conclusion

From our 45 days feeding trial, we conclude that fermented *Jatropha* protein concentrate can be included up to 20% in the diet for rohu without any detrimental effect on the growth and heamato-immunological status. Though the inclusion of 5% JPC did not elicit any adverse effects on the fish based on the parameter studied, however, a further long-term study is recommended. But FJPC can be considered as a potential protein source in *Labeo rohita* diet.

Conflict of interest

The authors declare that there is no conflict of interest.

Disclosure

The authors have nothing to disclose.

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