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Effect of ethanolic extract of *Mucuna pruriens* on growth, metabolism and immunity of *Labeo rohita* (Hamilton, 1822) fingerlings.

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Abstract
The effect of dietary ethanolic extract of *Mucuna pruriens* on growth, metabolism and haemato-immunological parameters of an Indian Major Carp, *Labeo rohita* fingerlings were studied. Fishes were fed with a diet containing four graded levels (0.0, 0.06, 0.08 and 0.1 g/100 g diet) of *Mucuna pruriens*. Net weight gain, specific growth rate (SGR) and food conversion ratio varied significantly ($p < 0.05$) improved in *Mucuna pruriens fed fish*. Still the highest weight gain and specific growth rate (SGR) were recorded in 0.06 g/100 gm *Mucuna pruriens* supplemented diet. The increase in digestive enzymes like protease, amylase and lipase supported the results of increase in growth at treatment level 0.06g/100gm.The metabolic enzymes like LDH, MDH and G6PDH levels were decreased in herbal extract supplemented groups. Whereas, the enzymes of protein metabolism were found increased in herbal extract supplemented group in comparison to control. Further, the haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb) and erythrocytes (RBC) were not significantly different in *Mucuna pruriens* supplemented diets as compared to control. A significant ($P < 0.05$) proliferation of the leukocytes (WBC) and improvement in respiratory burst activity. The results of this study proved the significant role of ethanolic extract of *Mucuna pruriens* in growth, metabolism and immunity defence mechanism of Labeo *rohita* fingerlings.

Keywords: *Mucuna pruriens*, Metabolism, haemato-immunology, rohu.

1. Introduction
Herbs have been widely used in veterinary and human medicine as they are not only safe for consumers but also widely available throughout Asia. The importance of herbal medicines in aquaculture has been advocated by Siddhuraju and Manian [1], Herken *et al*. [2] and Randhir *et al*. [3]. The use of herbs in diet has been viewed in a positive way in recent years by both scientists and consumers. Thus resulted in a push to procure foods with specific health benefits, such as functional foods. *Cynodon dactylon* (L.) ethanolic extract administered as feed supplement in *Catla catla* significantly increased the response of non-specific immunity [4]. *Plumbago rosea* in fish, *Catla catla* increased TEC, TLC, Increase in lymphocyte count, increase in Hb and serum protein level [5].The use of garlic in rainbow trout decreased mortality rate and increased immunity [6].Significantly enhanced primary and secondary antibody response and an inverse relationship was observed between the dose of leaf extract and the degree of immunostimulation in catla fed on *Ocimum sanctum* [7].
Traditionally, in India, the seeds of *M. pruriens* are used as a tonic and aphrodisiac for male virility. The pods are anthelmintic and seeds anti-inflammatory [8]. The seed powder has recently been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA. It is well known that dopamine is the brain neurotransmitter. The dopamine content in the brain tissue gets reduced because of its blockade of crossing over the blood brain barrier to reach the site of action. As L-DOPA is the precursor of dopamine, it crosses the barrier and gets converted into dopamine resuming the neurotransmission [9]. These important biological actions have led to the chemical investigations of *M. pruriens* seeds to isolate several fatty acids, amino acids besides L-DOPA [10]. The presence of alkaloids has also been reported with the tentative names like prurienine, prurieninine, prurienidine, or bases - P, Q, R, S, X etc. [11, 12]. Looking to the scientific and commercial importance of this underutilized legume, present study was designed to evaluate the effect of *Mucuna pruriens* seed extract on growth, metabolism and haemato-immunological parameters of Indian major carp, *Labeo rohita* fingerlings.

2. Material and Method

i) Experimental Design: The present experiment was conducted in eighteen cemented tanks of 3 m^3 capacity for 90 days. Healthy rohu, *Labeo rohita* fingerlings were obtained from fish seed production unit of MUPAT, Udaipur (India). Prior to the start of the experiment, the fish were fed with commercial feed for 7days to make the fish acclimatized to experimental environments. The healthy fingerlings of uniform size (14/18±0.00 g) were randomly distributed in six experimental groups each with three replicates following a complete randomized design. Each cemented tank (3x3x1 m size filled with 5 m^3 water) was stocked with 10 fish fingerlings. They were fed daily at 3.0% of their body weight for 90 days period.

2.1. Preparation of Extract

The seeds of *Mucuna pruriens* were procured locally and authentication was done by the expert of Botany Department (College of Science, MLS University, Udaipur, India). Thus the seeds procured were washed using tap water and then rewashed with distilled water to remove the dust. The seed were dried under shade for 7 days and seed coat was removed before grinding them to fine powder. Later, it was transferred into 5lit. Glass beaker and ethanol was added as solvent until the fine particles of the seed were completely soaked. The container was gently shaken for 72 h at every 1 h interval (until the colour of solvent became colourless) and the filtrate was vacuum concentrated to remove the moisture content [13]. The percentage of yield of extract from seed was around 20%. The dried extract powder was packed in sealed polythene bags and placed in deep freezer till further use.

2.2. Formulation of Experimental Diet

A basal diet having 35% crude protein was formulated using different ingredients (Table 1). The ingredients were powdered, thoroughly mixed and moistened with water to form dough. Thus, the dough prepared was placed in autoclave (121 ^{0}C temperature and 15l bs/cm^2 pressure) for 15 minutes. After cooling, graded levels of ethanolic extracts of *Mucuna pruriens* seeds were added to the basal diet at 0 (T1), 0.06(T2), 0.08(t3) and 0.1 g (T4)/100 g of basal diet. The feedstuffs were thoroughly mixed using a die of 8 mm diameter. The diets were air-dried at ambient temperature for 72 h, broken into small pellet sizes, packed in air-tight containers, labelled and stored.

Table 1: Ingredients (g/100gm dry matter) of the experimental diets for experiment.

Ingredients	T1	T2	T3	T4
Fishmeal	10	10	10	10
GNOC	44.96	44.96	44.96	44.96
Rice bran	21.52	21.42	21.22	20.82
Wheat flour	22.52	22.52	22.52	22.52
Mineral mixture (Agrimin)	1.0	1.0	1.0	1.0
Ethanolic extract of *Mucuna pruriens*	0.00	0.06	0.08	0.10
Moisture	8.25±0.016^a	8.31±0.023^b	8.56±0.029d	8.45±0.006c
protein	34.91±0.023^a	34.88±0.012a	35.04±0.017b	35.15±0.058b
ether extract	12.33±0.017^b	12.24±0.023a	12.49±0.012c	12.63±0.029d
carbohydrate	30.8±0.1^a	30.93±0.018a	30.64±0.024a	30.4±0.231a
Ash	13.71±0.006^d	13.64±0.012d	13.27±0.017a	13.37±0.029b
Energy(Kcal/gm)	431.93±0.005^b	431.445±0.006b	433.495±0.009d	434.435±0.003f

Mineral Mixture Agrimin forte-Nutritional value per kg.-Vit. A-7,00,000I.U., Vit.D3-70,000I.U., Vit.E-250 mg, Nicotinamide-1000 mg, Cobalt-150 mg, Copper-1200 mg, Iodine- 325 mg, Iron -1500 mg, Magnesium-6000 mg, Maganese-1500 mg,Potassium-100 mg,Selenium-10 mg, Sodium-5.9 mg, Sulphur-0.72%, Zinc-9600 mg, Calcium-25.5% and Phosphorus-12.75%. (supplied by Virbac Animal Health India Pvt. Ltd., Mumbai).

2.3. Growth Performance

The fish growth and nutrient utilization data were collected. Gross energy was calculated according to Jobling [14] with multiplier factors of carbohydrate, 4.1 kcal/g, protein, 5.4 kcal/g and lipids, 9.5 kcal/g. The following formulae were used to calculate, specific growth rate (SGR), food conversion ratio (FCR) and feed efficiency ratio (FER):

$$SGR = \frac{(\text{Log e Final wt.} - \text{Log e Initial Wt.})}{\text{Culture Period}} \times 100$$

$$FCR = \frac{\text{Total feed given}}{\text{Total weight gain}}$$

$$FER = \frac{\text{Total wet weight gain}}{\text{Total feed given}}$$

The proximate analysis

The proximate analysis of experimental feed and fish were done following the method of AOAC [15]. Feed composition was estimated before the initiation of experiments, while that of the experimental fish, the proximate composition was performed initially and at the termination of experiments.

2.4. Haematological Parameters

At the end of the 90 days feeding trial, fish were fasted for 24 hours immediately prior to blood sampling and five fish per tank were randomly chosen and anesthetized with tricaine methanesulfate (20 mg/L). The blood was extracted from the caudal vein and poured in Eppendorf tubes. One capillary of 50 microliter was filled and placed in blood analyser (Exigo Vet.) and result for RBC,MCV, HCT, HGB, PLT,WBC, MCHC, HCT, Lymphocytes and granulocyte were obtained.

2.5. Blood glucose

Five hundred micro litre (µl) of blood sample was deproteinised by mixing with 4.75 ml of zinc sulphate followed by addition of 4.75 ml of barium hydroxide [16]. The solution was mixed vigorously and filtered using a filter paper and the filtrate was collected in a dry test tube and 1 ml of alkaline copper sulphate was added to it. The test tubes were placed in a boiling water bath for 20 min. The

test tubes were then cooled to room temperature and 1 ml arsenomolybdate reagent was added. The absorbance was recorded at 540 nm against blank.

2.6. Nitroblue tetrazolium (NBT) assay

Nitroblue tetrazolium assay was done by the Secombs [17] as modified by Stasiacks [18]. Fifty micro litre of blood was placed into the wells of "U" bottom microtitre plates and incubated at 370 °C for 1 hour to facilitate adhesion of cells. The supernatant was removed and the loaded wells were washed three times in PBS. After washing, 50 µl of 0.2% NBT was added and was incubated for further 1 hr. The cells were then fixed with 100% methanol for 2-3 min and again washed thrice with 30% methanol. The plates were then air dried. Sixty microliters 2N potassium hydroxide and 70 microliters dimethyl sulphoxide were added into each well to dissolve the formazon blue precipitate formed. The OD of the turquoise blue coloured solution was then read in ELISA reader at 620 nm.

2.7.1. Serum protein

For separation of serum, blood samples were withdrawn from the caudal vein and transferred to Eppendorf tubes without anticoagulant. The blood samples were centrifuged at 3000 x g for 15 minutes and the supernatant serum was collected and stored at -200 °C until used. Serum protein was estimated by biuret method [19].

2.7.2. Albumin- globulin ratio

Albumin was estimated by bromocresol green binding method [20]. Whereas, the globulin was obtained by subtracting albumin values from total plasma protein. Albumin- globulin ratio was calculated following formula:

$$\text{A/G ratio} = \frac{\textit{Albumin}\ (g\%)}{\textit{Globuline}\ (g\%)}$$

2.7.3. Alanine & Aspartate amino transferase (sAST)

Serum Alanine & Aspartate amino transferase were measured by the auto-analyser MERCK Selectra Junior, Merck, Germany by using commercially available kit from Qualigens Diagnostics following standard protocols.

2.8. Functional immune response assays

2.8.1. Serum lysozyme

The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg ml-1 lyophilized *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate. Fifty µl of serum was added to 2 ml of the bacterial suspension and the reduction in the absorbance at 540 nm was determined after 0.5 and 4.5 minutes incubation at 220 °C. One unit of lysozyme activity was defined as a reduction in absorbance of 0.001 min-1.

2.9. Aspartate amino transferase (AST)

The Aspartate Amino Transferase (AST) activity was assayed in different tissue homogenates as described by Wooten [21]. The substrate comprised of 0.2 M D, L- aspartic acid and 2mM α-ketoglutarate in 0.05M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5ml of substrate was added. The reaction was started by adding 0.1ml of tissue homogenate. The assay mixture was incubated at 370 °C for 60 minutes. The reaction was terminated by adding 0.5 ml of 1 mM 2, 4 dinitrophenyl hydrazine (DNPH). In the control tubes. The enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 minutes with occasional shaking. Then 5 ml of 0.4 ml NaOH solution was added, the contents were thoroughly mixed. After 10 minutes, the OD was recorded at 540 nm against blank.

2.10 Lactate dehydrogenase (LDH)

The LDH activity was assayed by the method [22]. The total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340 nm at 30 sec interval. The enzymatic activity was expressed as units mg protein-1 min-1 at 250 ^{0}C where 1 unit was equal to Δ0.01OD min-1.

2.11 Malate dehydrogenase (MDH)

The MDH activity was assayed in different tissues by the method [23]. Total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1ml of phosphate buffer solution), 0.1 ml of tissue homogenate and 0.1 ml of freshly prepared oxaloacetate solution (2 mg oxaloacetate dissolved in 2 ml chilled distilled water). The reaction was started after addition of oxaloacetate solution as substrate. The OD was recorded at 340nm at 30 seconds interval for 3 minutes. The enzymatic activity was expressed as units/ mg protein/ min at 250 °C where 1 unit was equal to Δ0.01 OD/ min.

2.12 Digestive enzyme assays

Three important digestive enzymes like intestinal amylase, protease and lipase were assayed using standard protocols. Protease activity was determined by the casein digestion method [24]. Amylase activity was estimated using dinitro-salicylic-acid (DNS) method [34]. While, the lipase activity (EC 3.1.1.3) was assayed by the method [25].

2.12.1 Alkaline& Acid Phosphatase

Alkaline phosphatase (ALP) (E.C. 3.1.3.1) activity was determined by Garen and Levinthal [26]. The procedure adopted for the estimation of ALP activity was same as that for ACP activity estimation except that the buffer which comprised of 0.2 M Sodium Acetate and Acetic Acid, having pH 5.0. The ACP activity was expressed as nanomoles P-nitrophenol released / mg protein / minute at 37 °C.

2.13 Statistical Analysis

The data collected for fish growth parameters, blood analysis etc. were statistically analysed using SPSS (SPSS Inc., Chicago, IL, USA, version 16.0) programme for windows.). As such, standard error of mean, ANOVA, and Duncan's Multiple Range Test were calculated to know the significance of experimental results.

3. Result and Discussion

3.1 Proximate Composition of Diets

The proximate composition of the experimental diets fed to *L. rohita* fingerlings is given in the Table 1. The estimated crude

protein contents of the diets got varied from 34.91 to 35.15%. The lipid contents of the diets varied from 12.33 to 12.63% the proximate composition of the experimental diets shows significant difference ($p>0.05$) among the treatments. All the diets were isoproteinous and isocaloric. In the present study, the diets were maintained with crude protein content of 34.91 to 35.15 % which is supported by [27], who suggested the optimum protein requirement for Indian major carps between 30-45% as supported by [28].

3.2 Growth parameters

Fish growth is a complex process governed by many parameters like fish species, nutrient present in the feed, feed additives and rearing environment individually or in combination. The growth parameters like % weight gain, Food Conversion Ratio, Feed Efficiency Ratio and Specific Growth Rate are presented in Fig.1 and Fig.2. The %weight gain, FCR, FER and SGR of the treatment groups fed with ethanolic extract of *Mucuna pruriens* at different inclusion levels were significantly different ($p<0.05$) from the control group. Significantly higher values of % weight gain (162.09±0.711), FER (0.512±0.001) and SGR (1.465±0.001) were found in T-2 group and lowest in T-1 (control). The lowest value of FCR (1.953±0.006) was found in T-2 group and highest (2.292±0.010) in T-1 group. The effects of herbs have been studied on many aquatic animals. Improvement in the growth of fish has been reported by feeding herbs supplemented diet [29, 30, 31, 32, 33, 34, 35]. In the present study, the growth and nutrient utilization by fish got decreased with the increasing level of *Mucuna pruriens* extract inclusion in fish diets. This could probably be a result of persistent consumption of *Mucuna pruriens* extract which could retard animal growth rate. Siddhuraju and Becker [36] found similar results in common carp fed with *Mucuna pruriens*. They observed no significant difference in fish growth as compared to control and concluded that the sensitivity of common carp, to the antinutritional factors contained in the mucuna seed meal produced low growth performance at higher inclusion levels. Further, the studies of Siddhuraju and Becker [37] have shown that the use of processed mucuna seeds significantly improved the growth performance and feed utilization in tilapia compared with raw mucuna seed. Supplementation of *Gynostemma pentaphyllum*, a traditional Chinese herbal medicine, to grass carp feed resulted in increased weight gain, feed conversion efficiency and specific growth rate [38].

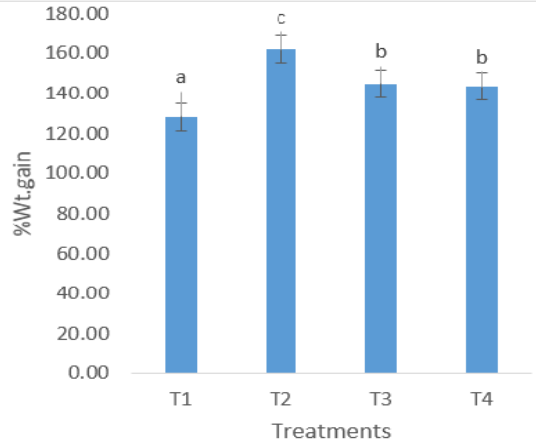

Fig 1: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on % weight gain of *Labeo rohita* fingerlings

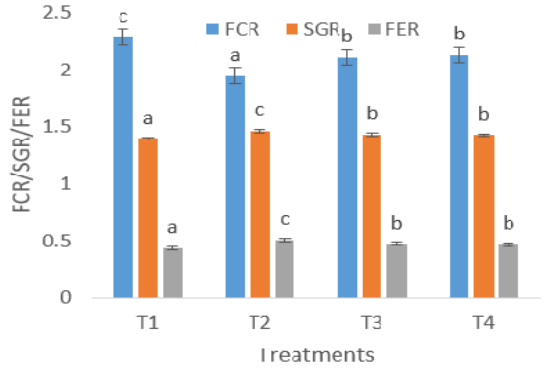

Fig 2: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Food Conversion ratio, Specific Growth Rate and Feed Efficiency Ratio of *Labeo rohita* fingerlings

3.3 Digestive Enzyme analysis

The digestive enzymes like protease, amylase, lipase, alkaline phosphatase and acid phosphatase activity in intestinal tissue of different experimental groups are given in Table 2. There is a significant effect on amylase activity due to *Mucuna pruriens* supplementation in the treatment groups. The highest amylase activity was observed in treatment group T-2 (26.452±0.32) and lowest in T-1(23.614±0.225). The protease activity was highest in treatment group T-2 (28.119±0.589) and lowest in treatment group T-1(22.948±0.455). The lipase activity was highest in treatment group T-2 (0.630±0.004) and lowest in T-1 (0.527±0.005). The enzyme activity of alkaline and acid phosphatase was significantly ($P<0.05$) different. The highest value of alkaline phosphatase was in treatment group T-2 (10.138±0.008) and lowest in T-1 (9.948±0.012). The acid phosphatase activity of treatment groupT-2 (4.073±0.018) was highest and lowest in treatment group T-1 (3.822±0.003). The level of digestive enzymes in fish may be influenced by type of feeding [39, 40, 41], biochemical composition of food and onset of sexual maturity [42].It is also known that age and stage of development significantly influence the digestive enzyme activities in different fish species [43, 44, 45, 46].

Amylase is one of the major carbohydrases which hydrolysis glycosidic bonds between sugar residues in large carbohydrate molecules. Amylase specifically breakdowns starch into glucose molecules. Low amylase activity in the carnivorous (with stomach) and high activity in omnivorous fishes (without stomach) is the general assumption [47, 48]. Proteases are digestive enzymes which hydrolyzes the peptide bonds between the adjacent amino acids in the proteins. Protease activities in intestine were higher than the hepatic protease activity, which was supported by the result of [49, 44]. Kumar *et al*., [49] reported functional efficacy of digestive proteases of catla (*Catla catla)*, rohu (*Labeo rohita)*, and silver carp (*Hypophthalmichthys molitrix)* total protease activity was higher in rohu followed by silver carp, and catla. Lipases

hydrolyzes the ester bonds among the fatty acids and glycerol in lipids. Alkaline phosphatase activity was reported to be an indicator of the intensity of nutrient absorption in enterocytes of fish [50, 51]. Abalaka *et al.*, [52] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed significant increases in the activity of alkaline phosphatase changed with increasing concentrations of both extracts.

Table 2: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Amylase, Protease, Lipase, Alkaline phosphatase and Acid phosphatase in Intestine of *Labeo rohita* fingerlings

Treatment	Amylase	Protease	Lipase	ALP	ACP
T1	23.614^{a}±0.225	22.948^{a}±0.455	0.527^{a}±0.005	9.948^{a}±0.012	3.822^{a}±0.003
T2	26.452^{c}±0.324	28.119^{c}±0.589	0.630^{c}±0.004	10.138^{d}±0.008	4.073^{d}±0.018
T3	25.849^{c}±0.339	26.183^{b}±0.589	0.585^{b}±0.007	10.052^{c}±0.007	3.970^{c}±0.007
T4	24.828^{b}±0.306	25.494^{b}±0.361	0.571^{b}±0.008	10.021^{b}±0.010	3.935^{b}±0.004

Amylase activity in micromole maltose released /min/gm protein; Protease activity in micromole tyrosine released/min/gm; Lipase activity in units/mg protein; Alkaline phosphatase activity in nano moles p-nitrophenol released /min/mg protein at 37 ˚C;Acid phosphatase activity in nano moles p-nitrophenol released /min/mg protein at 37 ˚C

3.4 Metabolic Enzymes

3.4.1 Enzymes of Carbohydrate Metabolism

The activity of MDH is also expected to increase during glycogenesis from amino acids. The activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities in muscle and liver of *L. rohita* are presented in Fig.3. The lactate dehydrogenase activity in liver was highest in treatment group T-1 (0.996±0.008) and lowest in T-2 (0.836±0.002). The lactate dehydrogenase activity in muscles was highest in treatment group T-1(0.855±0.004) and lowest in T-2 (0.703±0.002). The malate dehydrogenase activity was significantly (P<0.05) different. The highest malate dehydrogenase activity in liver was in treatment group T-1(0.471±0.007) and lowest in treatment group T-2 (0.277±0.003). The malate dehydrogenase activity in muscles was highest in treatment group T-1(0.386±0.004) and lowest in treatment group T-3 (0.233±0.010).

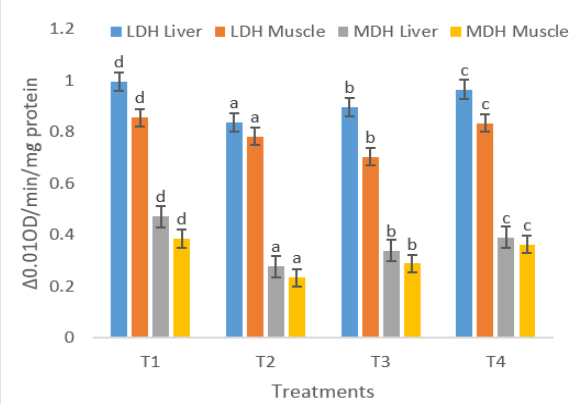

Fig 3: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH) in *Labeo rohita*

3.4.2 Enzymes of Protein Metabolism

The activities of aspartate amino transferase (AST/GOT) and alanine amino transferase (ALT/GPT) in liver and muscle of *L. rohita* fingerlings are presented in Table 3. The ethanolic extract of *Mucuna pruriens* showed a significant effect on these metabolic enzymes. The aspartate amino transferase activity in liver and muscles were significantly (P<0.05) different and in liver it was highest in treatment group T-2(9.306±0.356) and lowest in treatment group T-1 (6.733±0.335). In muscles, aspartate amino transferase activity was highest in treatment group T-2 (10.996±0.905) and lowest in treatment group T-1 (6.965±0.699). The alanine amino transferase activity was significant (P<0.05) and in liver it was highest in treatment group T-2 (3.756±0.014) and lowest in treatment group T-1(3.256±0.011). The alanine amino transferase activity in muscles was highest in treatment group T-2 (2.852±0.005) and lowest in treatment group T-1(2.357±0.011). Ananta *et al.*, [53] studied the effect of ethanolic extract of *Pedalium murex* fruit to ethylene glycol intoxicated rats and *Pedalium murex* reverted the levels of the liver and renal tissues from the damage and maintained the ACP, AST, ALP and ALT in renal and hepatic tissues. Abalaka *et al.*, [52] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed significant increases in aspartate amino transferase and alanine amino transferase activities.

Table 3: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in *Labeo rohita* fingerlings

Treatment	AST		ALT	
	Liver	Muscle	Liver	Muscle
T1	$6.773^{a}\pm0.335$	$6.925^{a}\pm0.699$	$3.256^{a}\pm0.011$	$2.357^{a}\pm0.011$
T2	$9.306^{c}\pm0.356$	$10.996^{c}\pm0.905$	$3.756^{d}\pm0.014$	$2.852^{d}\pm0.005$
T3	$7.889^{b}\pm0.378$	$8.883^{b}\pm0.384$	$3.464^{b}\pm0.013$	$2.460^{b}\pm0.011$
T4	$8.649^{bc}\pm0.292$	$10.905^{c}\pm0.207$	$3.558^{c}\pm0.014$	$2.654^{c}\pm0.014$

Aspartate transaminase activity in nano moles of oxaloacetate formed/min/mg protein; Alanine transaminase activity in nano moles sodium pyruvate released/min/mg protein.

3.5 Haemato-immunological Parameters

3.5.1 Blood count

The haematological responses like TEC count, TLC count, haemoglobin, haematocrit, MCV, MCH and MCHC values at the end of the experimental period are shown in Table 4. There was a significant ($P<0.05$) effect of ethanolic extract of *Mucuna pruriens* supplementation on these parameters. The highest value of total erythrocyte count (TEC) was in treatment group T-2 (1.32±0.01) and lowest in treatment group T-1(1.21±0.01). The total leucocyte count (TLC) was highest in treatment group T-1 (233.27±0.10) and lowest in treatment group T-4 (231.40±0.05). The haemoglobin content of treatment group T-2 (8.24±0.04) was highest and lowest in treatment group T-1 (6.90±0.03). Haematocrit (Hct) value of treatment group T-1(25.51±0.04) was lowest and treatment group T-2 (28.33±0.03) was highest. The mean cell volume was highest in treatment group T-3 (215.07±0.52) and lowest in treatment group T-1 (210.02±0.82). The mean corpuscular haemoglobin concentration (MCHC) was highest in treatment group T-2 (29.07±0.17) and lowest in treatment group T-1(27.06 ±0.09). The haemoglobin concentration got decreased with increase in concentration of the plant extracts similar to those reported in *C. gariepinus* to cassava effluents and tobacco (*Nicotina tobaccum*) leaf extracts [54, 55] and aqueous leaf extracts of *Lpidagnathis alopecuroides* [56] as compared to control, RBC values were significantly higher in experimental diet fed fish. Sahu *et al*., [57] have also reported higher RBC counts in *Labeo rohita* fingerlings fed with *Mangifera indica*. They explained this increase as an indication of enhanced cellular immunity. Chukwudi *et al*., [58] observed that WBC counts in rats administered with *Mucuna pruriens* increased significantly in comparison to control. This increase in WBC total count likely had been triggered off by the metabolic assault from alkaloid and/or phenol content in *Mucuna pruriens* [59]. The blood parameters such as MCV, MCH and MCHC are particularly important for the diagnosis of anemia in most of the animals. The MCV values decreased with increasing level of *Pedalium murex* extract inclusion levels in diet. Similar results were observed [29] and [60] for Tilapia fed with *A. paniculata* supplemented diet.

Table 4: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Haematological parameter in *Labeo rohita* fingerlings

Treatment	TEC[1]	TLC[2]	HGB[3]	HCT[4]	MCV[5]	MCH[6]	MCHC[7]
T1	$1.21^{a}\pm0.01$	$233.27^{d}\pm0.10$	$6.90^{a}\pm0.03$	$25.51^{a}\pm0.04$	$210.02^{a}\pm0.82$	$56.83^{a}\pm0.36$	$27.06^{a}\pm0.09$
T2	$1.32^{d}\pm0.01$	$232.55^{c}\pm0.06$	$8.24^{d}\pm0.04$	$28.33^{d}\pm0.03$	$214.17^{bc}\pm0.80$	$62.26^{d}\pm0.21$	$29.07^{c}\pm0.17$
T3	$1.25^{b}\pm0.01$	$231.90^{b}\pm0.07$	$7.31^{b}\pm0.03$	$26.83^{b}\pm0.05$	$215.07^{c}\pm0.52$	$58.62^{b}\pm0.18$	$27.26^{a}\pm0.15$
T4	$1.30^{c}\pm0.01$	$231.40^{a}\pm0.05$	$7.74^{c}\pm0.02$	$27.58^{c}\pm0.02$	$212.51^{b}\pm0.29$	$59.62^{c}\pm0.17$	$28.06^{b}\pm0.04$

[1]TEC=Total Erythrocyte count (10^6 cells/mm^3); [2]TLC=Total lymphocyte count (10^3cells/mm^3); [3]HGB=Haemoglobin (gm %); [4]Hct=Hematocrit (%); [5]MCV=Mean cell volume (fl); [6]MCH=Mean corpuscular hemoglobin (pg); [7]MCHC=Mean corpuscular Hemoglobin concentration (gm/dl)

3.5.2 Blood Glucose and Respiratory Burst Activity (NBT)

Blood glucose levels and the respiratory burst activity (NBT) of various experimental groups are presented in Table 5.There was a significant effect ($p<0.05$) of *Mucuna pruriens* supplementation on the blood glucose level and the respiratory burst activities of the different experimental groups. The highest value of blood glucose was in the group T-1(51.620±0.307) and lowest in treatment group T-2(35.640±0.285).The respiratory burst activity was observed highest in treatment group T-2(0.176±0.013) and lowest in treatment group T-1 (0.125±0.006).

Blood glucose concentration in blood serum got reduced significantly in fish fed on diets containing different sources of *Allium sativum*. This condition was attributed to improve the antioxidant system in cells of pancreas to produce insulin [61]. These results got conformed to those of [62, 63] who found that feeding mice with garlic induced significant decrease of serum glucose levels. Lower levels of plasma glucose in fish have also been reported in the assessment of biochemical effects of *Allium sativum* [64]. Bhaskar *et al*., [65] reported that aqueous extract of the seeds of Mucuna pruriens significantly reduced the blood glucose levels after an oral glucose load and oral administration of seed extract. Herbal medicine extracts can also enhance phagocytosis in various fish species [66, 67]. Their phagocytic activity is a primitive defence mechanism [68] and an important characteristic of the non-specific immune system [69].

Table 5: Effect of dietary supplementation of ethanolic extract of *Mucuna pruriens* on Serum total protein, albumin, globulin, A/G ratio, aspartate transaminase, alanine transaminase, lysozyme activity, Glucose and NBT in *Labeo rohita* fingerlings

Treatment	T1	T2	T3	T4
Total protein	$5.523^{a}\pm0.226$	$6.253^{b}\pm0.107$	$8.327^{c}\pm0.017$	$5.807^{ab}\pm0.111$
Albumin	$2.530^{b}\pm0.130$	$1.917^{a}\pm0.049$	$3.177^{c}\pm0.106$	$2.240^{b}\pm0.049$
Globulin	$2.993^{a}\pm0.096$	$4.337^{b}\pm0.081$	$5.150^{c}\pm0.402$	$3.567^{a}\pm0.077$
A/G ratio	$0.844^{c}\pm0.016$	$0.442^{a}\pm0.012$	$0.627^{b}\pm0.065$	$0.628^{b}\pm0.086$
AST	$59.713^{c}\pm0.273$	$63.600^{d}\pm0.306$	$48.833^{a}\pm0.221$	$55.563^{b}\pm0.279$
ALT	$22.117^{a}\pm0.104$	$30.490^{d}\pm0.321$	$27.443^{b}\pm0.237$	$28.870^{c}\pm0.321$
Lysozyme	$4.022^{a}\pm0.004$	$8.918^{d}\pm0.004$	$6.736^{b}\pm0.006$	$8.418^{c}\pm0.006$
Blood Glucose	$51.620^{d}\pm0.307$	$35.640^{a}\pm0.285$	$45.233^{b}\pm0.252$	$40.040^{c}\pm0.305$
NBT	$0.125^{a}\pm0.006$	$0.176^{c}\pm0.013$	$0.146^{ab}\pm0.004$	$0.163^{bc}\pm0.005$

Total protein (gm %); Albumin (gm %); Globulin (gm %); Aspartate transaminase activity in nano moles of oxaloacetate formed/min/mg protein; Alanine transaminase activity in nano moles sodium pyruvate released/min/mg protein; Lysozyme (unit ml^{-1}); Blood Glucose (mg/dl); NBT-Respiratory burst activity (OD at 540 nm)

3.5.3 Serum Parameters

Serum parameters, total protein, albumin, globulin, A/G ratio, lysozyme, aspartate amino transferase (AST) and alanine amino transferase activity of the experimental groups supplemented with ethanolic extract of *Mucuna pruriens* are shown in Table 5.The highest total protein value was in treatment group T-3(8.327±0.017) and lowest in treatment group T-1(5.523±0.226). The serum albumin content was highest in treatment group T-3 (3.177±0.106) and lowest in treatment group T-2(1.917±0.049). The globulin content of treatment group T-3 (5.150±0.402) was highest and lowest in T-1(2.993±0.096). The albumin globulin ratio (A/G ratio) was highest in treatment group T-1(0.844±0.016) and lowest in treatment groupT-2 (0.442±0.012). Serum aspartate amino transferase (AST/GOT) and alanine amino transferase activity (ALT/GPT) were significantly ($P<0.05$) different in all treatment groups. The highest aspartate amino transferase activity was in treatment group T-2 (63.600±0.306) and lowest in treatment group T-3 (48.833±0.221). The alanine amino transferase activity was highest in treatment group T-2 (30.49±0.321) and lowest in treatment group T-1(22.117±0.104). The lysozyme activity in treatment group T-2 (8.918±0.004) was highest and treatment group T-1 (4.022±0.004) was lowest.). Abalaka *et al*., [52] mentioned that *Clarias gariepinus* adults exposed to aqueous and ethanolic extracts of *Parkia biglobosa* pods showed plasma total proteins concentrations increased with increasing extracts concentrations in fish exposed to ethanolic extracts. The results of the present study and specially the improvement in the lysozyme level in *Mucuna pruriens* supplemented diet fed fish found support from the results of crucian carp, large yellow croaker [70] and the common carp [71] when the fish were fed with various herbal extracts that included *Eclipta alba, Radix astragalin seu* Hedysari and *Radix angelicae sinensis*..

4. Conclusion

Based on these results and discussions, it is concluded that *Mucuna pruriens* extract supplemented diet has significant role in improving growth of *L. rohita* besides its ability to enhance metabolism and immunity of the fish. The optimum dose (0.06 gm/100 gm diet) in the feed of *L. rohita* need to be further tested under field condition so that the *Mucuna pruriens* may be recommended for the commercial aquaculture.

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