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Growth, Metabolism and Haematological parameters of *Labeo rohita* (Hamilton, 1822) fingerlings fed with herbal supplemented diet

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Abstract
The effect of herb supplemented diet on growth, metabolism and Haemato-immunological parameters of an Indian Major Carp, *Labeo rohita* fingerlings was studied. Fishes were fed with a diet containing four graded levels (0.0, 0.06, 0.08 and 0.1 g/100g diet) of herbal combination of *Mucuna pruriens* and *Pedalium murex* (1:1). The supplemented diet significantly (p<0.05) improved the growth, specific growth rate (SGR) and food conversion ratio (FCR) as compared to control. Still the highest weight gain and specific growth rate (SGR) were recorded with 0.06g per 100g herbal supplemented diet. The increase in digestive enzymes like protease, amylase and lipase supported the results of increased growth at treatment level 0.06g/100. The levels of metabolic enzymes (i.e. LDH and MDH) in lever and muscles were significantly (p<0.05) decreased in treatments as compared to control. However, the enzymes of protein metabolism were significantly (p<0.05) increased in comparison to control. Similarly haematological parameters such as packed cell volume (PCV), haemoglobin content (Hb) and erythrocytes (RBC) were significantly (p<0.05) different in treatments and control. A significant (p<0.05) proliferation of the leukocytes (WBC) was recorded in herbal supplemented diet. The results of this study proved a significant role of herbal combination of *Mucuna pruriens* and *Pedalium murex* on growth, metabolism and immune defence mechanism of *Labeo rohita* fingerlings.

Keywords: Mucuna pruriens, pedalium murex, herbal combination, metabolism, haemato-immunology, *Labeo Rohita*

Introduction
Herbal preparations have been widely used in veterinary and human medicine as they are not only safe for consumers but also widely available throughout Asia. Traditionally, in India, the seeds of *Mucuna pruriens* are used as a tonic and aphrodisiac for male virility. The pods are reported to be anthelmintic and seeds anti-inflammatory. The seed powder has recently been found to show the anti-Parkinsonism effects which are probably due to the presence of L-DOPA [1]. 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 [2]. Considering this biological action, the isolation of several fatty acids, amino acids and L-DOPA from *M. pruriens* was initiated [3].

Pedalium murex (Gokshura) is a rich source of flavonoids, sapogenin, sterol and soluble proteins [4]. In Indian Ayurvedic medicine system, gokshura is mainly used as analgesic and antipyretic drug [5, 6]. Pharmacologically, the gokshura plant has been investigated for antiulcerogenic, nephroprotective, hypolipidemic, aphrodisiac, antimicrobial and insecticidal properties [9-12].

The importance of herbal medicines in aquaculture has been advocated by Siddhuraju and Manian [13-15]. Effect of Mucuna *M. pruriens* on *Labeo rohita* fingerlings have been studded by Ojha *et al* [16, 17], and enhanced growth rate, immunity, metabolism of the experimental fish was reported. Similar results had also been observed in *L. rohita* feed on ethanolic extract of *Pedalium murex* [18, 19]. Considering the scientific and biological importance of both these herbs, the present study was designed to evaluate the effects of herbal combination of

Mucuna pruriens and *Pedalium murex* seed (ethanolic extract) to study the synergetic effects of these herbs on growth, metabolism and haematological parameters of an Indian major carp, *Labeo rohita* fingerlings.

2. Material and Methods

Experimental Design: The present experiment was conducted in 12 cemented tanks of 3 m^3capacity for 90 days. Healthy rohu, *Labeo rohita* fingerlings were obtained from fish seed production unit of MUPAT, Udaipur (India). Initially, the fish were fed with commercial feed for 7days to make the fish acclimatized to experimental conditions. The healthy fingerlings of uniform size (16 ± 2g) 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 fingerlings. They were fed daily at 3.0% of their body weight for a period of 90 days.

Preparation of Herbal Extract: The seeds of *Mucuna pruriens* and *Pedalium murex* were procured locally and authentication was done by the expert of Botany Department (College of Science, MLS University, Udaipur, India). The seeds procured were washed using tap water and rewashed with distilled water to remove the dust. The seeds were dried under shade for 7 days, seed coat was removed and finally grinded to fine powder. The seed powder was transferred to a five litre 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 color of solvent became colorless) and the filtrate was vacuum concentrated to remove the moisture content [20]. The percentage of yield of extract from seed was around 20% in case of *Mucuna pruriens*, whereas in case of *Pedalium murex* it was only 8.5%. The dried extract powder was packed in sealed polythene bags and placed in deep freezer till further use.

Formulation of Experimental Diet: Isoprotein 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 ˚C temperature and 15 lbs/cm^2 pressure) for 15 minutes. After cooling, graded levels of ethanolic extract combination (1:1) of *Mucuna pruriens* and *Pedalium murex* seeds were added to the basal diet at 0g (T1), 0.06g (T2), 0.08g (T3) and 0.1g (T4) per 100g of basal diet. The ingredients were thoroughly mixed and diets were prepared by using a die of 8 mm diameter. The feeds were air-dried at ambient temperature for 72 h, broken into small pellet sizes, packed in air-tight containers, labelled and stored at 4^0 C in refrigerator.

Growth Performance: The fish growth and nutrient utilization data were collected. Gross energy was calculated according to Jobling [21] with multiplier factors of carbohydrate-4.1 kcal/g, protein-5.4kcal/g and lipids-9.5kcal/g. The following formulae were used to calculate, specific growth rate (SGR), food conversion ratio (FCR) and feed efficiency ratio (FER):

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

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

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

Proximate Analysis: The proximate compositions of experimental diets were determined by standard methods of AOAC [22].

Blood Sampling: Fish were anesthetized with CIFECALM (50 µl/l) [23], an anaesthetic herbal formulation containing natural alcoholic extracts of *Eugenia caryophyllata* and *Mentha arvensis* (developed by CIFE). Blood was withdrawn from the caudal vein with a 2ml syringe and a 26-G needle within 2 min after capture and immediately transferred to dry Eppendorf tubes. The tubes were allowed to stand in a tilted position for 1 h to collect the serum, which was subsequently used to analyze serum glucose, alanine amino transferase (ALT), and aspartate amino transferase (AST). Pure blood was taken in a capillary of 50 microliter which was directly placed in Automatic Blood Analyzer (Exigo Vet.) and results for RBC, MCV, HCT, HGB, PLT, WBC, MCHC, HCT, Lymphocytes and granulocyte were obtained.

Enzyme Assays

Lactate Dehydrogenase (LDH)

Lactate dehydrogenase activity was assayed by the method of Wroblewski and Ladue [24]. 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 (2mg 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 sodium pyruvate. The OD was recorded at 340 nm at 30 second interval. The enzymatic activity was expressed as units mg $protein^{-1}$ min^{-1} at 25 ˚C where 1 unit was equal to Δ0.01 OD min^{-1}.

Malate Dehydrogenase (MDH)

The MDH activity was assayed in different tissues by the method of Ochoa [25]. Total 3 ml of reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml NADH solution (2 mg NADH dissolved in 1 ml 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 340 nm at 30 seconds interval for 3 minutes. The enzymatic activity was expressed as units mg $protein^{-1}min^{-1}$ at 25 ˚C where one unit was equal to Δ0.01 OD/min.

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 [26]. Amylase activity was estimated using dinitro-salicylic-acid (DNS) method and lipase activity (EC 3.1.1.3) was assayed by the method of Cherry and Crandall [27].

Statistical Analysis: Data collected were statistically analyzed following one-way ANOVA. The ANOVA results were further tested to find the significant differences between treatments and control using Duncan's multiple comparison

procedures. All statistical analyses were performed using SPSS 16.0 for Windows.

Result and Discussion

Proximate Composition of Diets: The proximate composition of experimental diets fed to *L. rohita* fingerlings is given in Table 1. The estimated crude protein contents of the diets varied from 34.96 to 35.15%. The lipid contents of the diets varied from 12.02 to 12.63% significant difference ($p<0.05$) in proximate compositions of diets was noticed. All the diets were Isoprotein and isocaloric. In the present study, the diets were maintained with crude protein content of 34.96 to 35.15% which is optimum for carps Renukaradhya and Varghese [28]. They suggested the optimum protein requirement for Indian major carps between 30-45%.

Growth Performance: Fish growth is a complex process governed by many parameters like fish species, nutrient present in the diet, feed additives and rearing environment individually or in combination. The growth parameters like weight gain, FCR, FER and SGR are graphically represented in Fig.1. The per cent weight gain, FCR, FER and SGR of the treatment group fed with ethanolic extract of *Pedalium murex* and *Mucuna pruriens* combination at different inclusion levels were significantly different ($p<0.05$) as compared to control group. Significantly higher values of per cent weight gain (167.22 ± 1.185%); FER (0.407 ± 0.001) and SGR (1.474 ± 0.002) were found in T2 treatment and lowest in T1 (control). The lowest value of FCR (2.461 ± 0.011) was found in T2 group and highest in treatment group T1 (2.907 ± 0.020). The effects of herbs have been studied on many aquatic animals. Enhanced growth of fish have been reported with the supplementation of herbs in deit [16-19, 29-34]. In the present study, the growth and nutrient utilization by fish got decreased with the increasing level of herbal ethanolic extract inclusion in fish diets. This could probably be a result of persistent consumption of ethanolic extract combination of *Mucuna pruriens* and *Pedalium murex* which could retard animal growth rate. Ojha *et al* [16-19] found similar results in *Labeo rohita* fed with *Mucuna pruriens* and *Pedalium murex*. They observed significant difference in fish growth as compared to control and concluded that the sensitivity of *Labeo rohita*, to the persistent use of herbal extract resulted in low growth performance at higher inclusion levels. However, the 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 [34].

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
Combination of ethanolic extract of *Mucuna pruriens* and *Pedalium murex*	0.00	0.06	0.08	0.10
Moisture	8.35 ± 0.016^{a}	8.44 ± 0.023^{b}	8.42 ± 0.029^{d}	8.45 ± 0.006^{c}
protein	34.96 ± 0.023^{a}	35.09 ± 0.012^{a}	35.03 ± 0.017^{b}	35.15 ± 0.058^{b}
ether extract	12.23 ± 0.017^{b}	12.02 ± 0.023^{a}	12.56 ± 0.012^{c}	12.63 ± 0.029^{d}
carbohydrate	39.15 ± 0.1^{a}	39.27 ± 0.018^{a}	39.07 ± 0.024^{a}	38.85 ± 0.231^{a}
Ash	13.66 ± 0.006^{c}	13.62 ± 0.012^{d}	13.34 ± 0.017^{a}	13.37 ± 0.029^{b}
Energy(Kcal/gm)	430.11 ± 0.005^{b}	429.17 ± 0.006^{a}	433.26 ± 0.009^{d}	433.56 ± 0.003^{c}

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

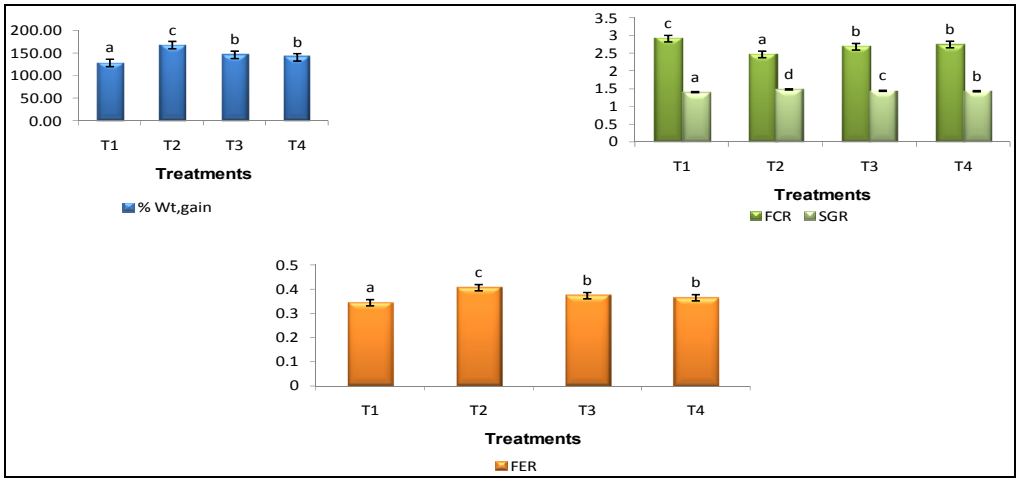

Fig 1: Effects of dietary supplementation of ethanolic extract of *Pedalium murex* and *Mucuna pruriens* (1:1) on growth parameters of *Labeo rohita* fingerlings.

Digestive Enzyme Analysis

The digestive enzymes like protease, amylase, lipase, alkaline phosphatase and acid phosphatase activity in intestinal tissue of experimental fish are given in Fig.2. There is a significant effect on amylase activity due to *Pedalium murex* and *Mucuna pruriens* combination supplementation in the treatments. The highest amylase activity was observed in treatment T2 (27.821 ± 0.663) and lowest in T1 (23.124 ± 0.541). The protease activity was highest in treatment T2 (28.488 ± 0.877) and lowest in treatment T1 (21.790 ± 0.344). The lipase activity was highest in treatment T2 (0.589 ± 0.008) and lowest in T1 (0.526 ± 0.005).

The level of digestive enzymes in fish may be influenced by type of feeding [35-37] biochemical composition of food and onset of sexual maturity [38]. It is also known that age and stage of development significantly influence the digestive enzyme activities in different fish species [39-41].

Amylase is one of the major carbohydrases which hydrolyzes 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 [42, 43]. Proteases are digestive enzymes which hydrolyzes 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 Kumar *et al* [44, 39]. Kumar *et al* [44] 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. Lipase 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 [45, 46]. Abalaka *et al* [47] 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 the extracts

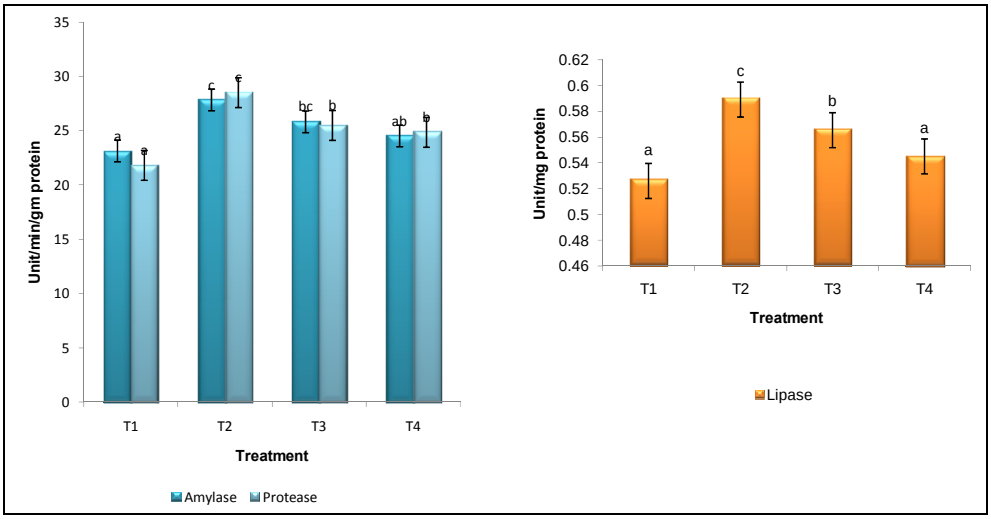

Fig 2: Effects of dietary supplementation of ethanolic extract of *Pedalium murex* and *Mucuna pruriens* (1:1) on Amylase, Protease, Lipase, in Intestine of *Labeo rohita* fingerlings

Metabolic Enzymes

Enzymes of Carbohydrate Metabolism: The activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) in muscle and liver of *L. rohita* are presented in Fig.3. The lactate dehydrogenase activity in liver was highest in treatment T1 (0.976 ± 0.004) and lowest in T2 (0.698 ± 0.003). The lactate dehydrogenase activity in muscles was highest in treatment T1 (0.911 ± 0.003) and lowest in T2 (0.652 ± 0.003). The malate dehydrogenase activity was significantly (P<0.05) different in muscles and liver of experimental fish. The highest malate dehydrogenase activity in liver was in treatment T1 (0.449 ± 0.005) and lowest in treatment T2 (0.225 ± 0.004). The malate dehydrogenase activity in muscles was highest in treatment T1 (0.402 ± 0.003) and lowest in treatment T3 (0.196 ± 0.005). Similar result were also observed by Ojha *et al* [18, 19].

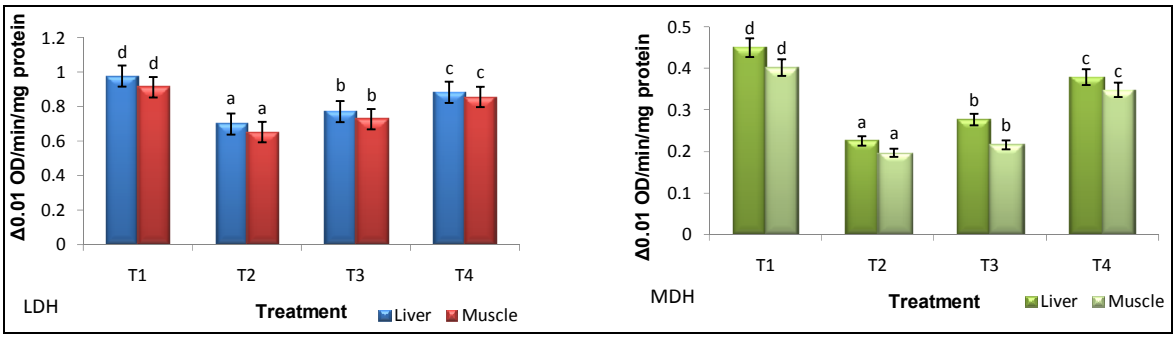

Fig 3: Effects of dietary supplementation of ethanolic extract of *Pedalium murex* and *Mucuna pruriens* (1:1) on Lactate dehydrogenase (LDH) and Malate dehydrogenase (MDH) in *Labeo rohita* fingerlings

Haemato-Immunological Parameters

Blood Count: The haematological responses of herbal diet on TEC count, TLC count, haemoglobin, haematocrit, MCV, MCH and MCHC of the experimental fish are shown in Table 2. There was a significant (P<0.05) effect of experimental diets on haematological counts. The highest value of total erythrocyte count (TEC) was in treatment T2 (1.33 ± 0.02) and lowest in treatment T1 (1.22 ± 0.01). The total leucocyte count (TLC) was highest in treatment group T1 (235.59 ± 0.18) and lowest in treatment group T2 (232.42 ± 0.26). The haemoglobin content of treatment T2 (8.36 ± 0.06) was highest and lowest in treatment T1 (6.84 ± 0.04). Haematocrit (Hct) value of treatment T1 (25.31 ± 0.05) was lowest and treatment T2 (28.50 ± 0.08) was highest. The mean cell volume was highest in treatment T2 (213.68 ± 1.25) and lowest in treatment T1 (206.64 ± 0.78). The mean corpuscular haemoglobin concentration (MCHC) was highest in treatment T2 (29.33 ± 0.27) and lowest in treatment T1 (27.02 ± 0.12).

In the present study, reduced haemoglobin content with increased plant extracts supplementation level was noticed. Similar results were also reported in *C. gariepinus* with cassava effluents and tobacco (*Nicotina tobaccum*) leaf extracts supplementations [48, 49]. While, feeding with aqueous leaf extracts of *Lepidagathis alopecuroides*, the RBC values were significantly higher in experimental diet fed fish than control [50]. Sahu *et al* [51] 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* [52] 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* [53]. 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 by Ojha *et al* [18, 19] and Suresh and Amolkumar [54] for Tilapia fed with *A. paniculata* supplemented diet.

Table 2: Effect of dietary herbal extract combination supplementation on Haematological parameters in *Labeo rohita* fingerlings

Treatment	TEC[1]	TLC[2]	HGB[3]	HCT[4]	MCV[5]	MCH[6]	MCHC[7]
T1	1.22^{a}±0.01	235.59^{d}±0.18	6.84^{a}±0.04	25.31^{a}±0.05	206.64^{a}±0.78	55.83^{a}±0.38	27.02^{a}±0.12
T2	1.33^{d}±0.02	232.42^{a}±0.26	8.36^{d}±0.06	28.50^{d}±0.08	213.68^{b}±1.25	62.67^{c}±0.30	29.33^{c}±0.27
T3	1.27^{b}±0.01	234.62^{b}±0.22	7.44^{b}±0.02	26.93^{b}±0.04	212.10^{b}±0.21	58.63^{b}±0.32	27.64^{b}±0.13
T4	1.29^{c}±0.01	235.18^{c}±0.35	7.64^{c}±0.02	27.48^{c}±0.03	212.29^{b}±0.35	59.00^{b}±0.21	27.79^{b}±0.04

Data expressed as Mean ± SE (n=6)

Mean in the same column sharing different superscript are significantly different (P<0.05)

[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=Haematocrit (%)

[5]MCV=Mean cell volume (fl)

[6]MCH=Mean corpuscular haemoglobin (pg)

[7]MCHC=Mean corpuscular Haemoglobin concentration (gm/dl)

Conclusion: Based on these results and discussions, it is concluded that *Pedalium murex* and *Mucuna pruriens* combination (1:1) extract supplemented diet has significant role in improving growth of *L. rohita.* Besides, it has ability also enhance metabolism and immunity of the fish. The optimum dose (0.06gm/100gm diet) in the feed of *L. rohita* need to be further tested under field condition so that the *Pedalium murex* and *Mucuna pruriens* combination supplementation may be recommended for the commercial aquaculture.

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