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Effect of partial replacement of fishmeal with aquatic weed *Pistia stratiotes* meal on growth, biochemical composition, haematological parameters and digestive enzymes in Indian major carp *Labeo rohita* (Hamilton, 1822)

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Abstract
80 days experiment was conducted to determine the growth, biochemical, haematological and digestive enzyme performance of *Labeo rohita* (1.25± 0.12 g), fingerlings fed with Water Lettuce, *Pistia stratiotes* young leaf meal. The fingerlings were fed with a control (0% *P. stratiotes*) and five different experimental diets, containing 10%, 20%, 30%, 40% and 50% of *P. stratiotes* meal in place of fish meal as protein source and named as P0, P10, P20, P30, P40 and P50 respectively. There was a significant difference (P<0.01) in growth, biochemical, haematological parameters and digestive enzyme activities of different treatment groups. Maximum weight gain, specific growth rate and better feed conversion were noticed in P30. Maximum protein and fat accretion were witnessed in P30 group. These results clearly showed that *L. rohita* fed with diet 30% *P. stratiotes* meal in the diet perform better than rest of the experimental groups. Hence, this study proved that although fishmeal is non-replaceable, but can be supplemented with *P. stratiotes* meal up to an optimum level (30%) to produce cost effective fed for *L. rohita.*

Keywords: *Labeo rohita, Pistia stratiotes*, growth, haematological, digestive enzymes

1. Introduction
Aquaculture produce plays a vital role in providing affordable high quality protein all around the world. The Indian major carps *Catla catla, Labeo rohita* and *Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production (ICLARM 2001) [16]. Fish protein has a high nutritional value due to a well balanced amino acid profile, ample amounts of polyunsaturated fatty acids (PUFA) as well as a number of vitamins and minerals (Edwards 1997) [11].

Traditional carp farming, mainly depends on primary production for its growth. Further, the growth can be accelerated through provision of supplementary feed to sustain the increased demand for aquaculture produce. Protein is the first nutrient on consideration while formulating a feed. Dietary protein is the main source of nitrogen and essential amino acids in animals. As protein is the costliest component used in artificial feeds, it is necessary to ascertain quantitative requirement in diet in order to reduce the cost of feeds (Ranjit Singh and Asha Dhawan 1996) [29].

The traditional feed mixture employed in the culture of Indian Major Carp (IMC) is unbalanced. Therefore, an urgent need to develop low-cost, nutritionally balanced IMC diets that can support increased production levels. The reduced availability as well as the escalating cost of fish meal has necessitated the need to identify suitable cost effective alternatives to fish meal. Replacement of fish meal with a complex mixture of plant protein might reduce the exposure of fish to individual anti-nutritional factor and improves growth performance (Borgeson 2006) [6]. On other hand green plants have long been recognized as the cheapest and most abundant potential source of protein because of their ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials (Fasuyi and Aletor, 2005) [12]. A Lot of work has been done to include many of the un conventional protein sources to be used in animal or fish feed but use of aquatic plant has given little emphasis. In this connection aquatic weeds or plant fodder, rich in protein may be considered as the

alternative protein sources in the aqua feed (Chiayvareesajja *et al.,*1989 ; Hasan *et al*., 1990; Keshavanath *et al*., 1990; Mbagwu *et al*., 1990) [9, 14, 20, 25]

Blood is a complex fluid containing a large variety of dissolved suspended organic an inorganic substances (Stewart, 1991) [36] or specialized circulating tissues and cells suspended in the intra cellular fluid substance (Dellman and Brown 1976) [10]. Haematological characteristics are an important tool that can be used to understand as an effective and sensitive index to monitor physiological and pathological changes in fishes. And also environmental and physiological factors are known to influence fish haematology, these include stress due to handling,

transporation, sampling, age and sex. Generally, both the biochemical and haematological blood components are influenced by the quantity and quality of feed and also the level of anti-nutritional elements or factors present in the feed (Akinmutimi, 2004) [1] and also food and feeding habits of fish related to digestive enzymes. Therefore, we investigated the effects of partial replacement of *P. stratiotes* on growth, biochemical composition, haematological parameters and digestive enzymes response of *L. rohita.*

2. Materials and Methods

2.1 Experimental fish: *L. rohita* (Hamilton, 1822) (1.23 ±0.008) was selected for the present experiment. The reason of its selection was that, it has high growth potential, coupled with high consumer preference and easy availability. Rohu is most important freshwater species cultured in India, Bangladesh and other adjacent countries in the region. Considering its importance in the culture system emphasis has also been given to its genetic improvement through selective breeding in India.

The experimental animal *L. rohita* were collected from Sabari fish farm, Tirunelveli (Dist) Tamilnadu, India. And then were immediately transported to the laboratory conditions. These fishes were acclimatized to the lab condition for a month. During acclimatization, the animals were fed with

dried pellets of 20% protein diet.

2.2 Experimental diet

For the experimental supplementary feed, *P. stratiotes* young leaves were added along with chosen ingredients. The fresh colonies of *P. stratiotes* were collected from the local pond, Tuticorin district, Tamilnadu, India. And thoroughly washed to remove dirt. Then dried at room temperature for 1 week. After that, these samples were powdered. Six dry diets were prepared in which fishmeal was replaced with *P. stratiotes* at 0%, 10%, 20%, 30%, 40% and 50% and named as P0, P10, P20, P30, P40 and P50 respectively.

2.3 Feed preparation

Feed formulation was done according to (Hardy 1980) [13] and 35% protein diet was prepared for experimental use. Test diets were prepared using ingredients like fishmeal, ground oil cake, rice bran, cod liver oil and vitamins and mineral mix. The dried and powdered ingredients were blended at first to make a homogenous mixture. Subsequently mixed with suitable level of dried

P. stratiotes leaf meal (0%, 10%, 20%, 30%, 40% and 50%) with an aliquot of boiled water and them steam cooked for 15-20 minutes in pressure cooker. After, moderate cooking pellets (2mm) were prepared with a hand operated pelletizer and dried in sunlight. After drying diets were separately stored for experimental use.

For the experiment *L. rohita* (1.23 ±0.008) were collected from the stock. Healthy and active fishes were divided into six groups and offered with different levels of *P. stratiotes* diet. Each group consisting of 25 individuals was reared in circular cement tank containing 100*l* of water (width: 58.5cm; height: 40cm; 120l capacity). Triplicates were maintained for each *P. stratiotes* diet. The tank was filled with dechlorinated well water (Temperature 28 ± 0.3 ºC; pH 7.6 ± 0.1; salinity 0.25± 0.2 ppt; water hardness 180 ± 4.48mg $CaCO_3$ l^{-1}; Do 4.03 ± 0.75 ml O_2 l^{-1}). This study was conducted for 80 days. They were fed at 5% body weight twice daily morning and evening at equal ration. Every day the uneaten feed and faecal matter were siphoned thoroughly. The growth was assessed at the interval of 20 days.

2.4 Sample collection and Analyses

At the beginning and end of the feeding trial, all fish from each replicate were weighed and counted for calculation of weight gain, specific growth rate, feed conversion ratio. At the end of the feeding period five fishes from each replicate were sacrificed for haematological, biochemical and enzymatological assays. Blood was collected from the caudal peduncle with the aid of 1 ml syringe fitted with 26 gauge needle without any anticoagulant for haematological studies. For quantitative estimations of RBC and WBC, a method originally devised by Yokoyoma (1947) [38] was employed. Shali-Hellize haemoglobinometer was used to determine the haemoglobin content from the blood. The muscle samples devoid of bones and analyzed for proximate composition. Crude protein was measured by (Lowry *et al.,* 1951) [23], crude lipid was estimated by the (Bradgon method 1951) [7], ash content performed by (Paine 1964) [27] and energy was determined by (Karzinkin and Tarkovskaya 1964) [19] method. The foregut, midgut and hindgut samples were collected from each replicate and homogenized separately with distilled water using mechanical dispenser. The homogenate was centrifuged at 4000 rpm for 15 minutes at 4ºC using high speed refrigerated centrifuge (Remi model K=II) Place to prepare 10% homogenate. The clear supernatant was used as the crude enzyme for subsequent assay. Amylase activity was measured by (Bern field, 1955) [5], protease was measured by (Jancy 1976) [17] and lipase was determined by (Teitz and Friedrick 1966) [37].

2.5 Stastical analysis

All data were expressed as the mean ± SD. All data of groups were analysed for significant differences by (ANOVA) using Microsoft Excel-2007 and significance difference was set up at $P<0.01$.

3. Results

P. stratiotes leaf meal was a good source of crude protein and crude lipid. The table (1) presents proximate composition of *P. stratiotes* leaf of protein, lipid, ash, fibre and nitrogen free extract. The table (2) showed the experimental diets were prepared using ingredients of fish meal, ground nut oil cake, rice bran, cod liver oil and vitamin and mineral mixture. The proximate composition of the six diets formulated for the feeding trial is presented in table (3) showed the crude protein content ranged between 25.85 and 35.44% with higher level in P 30 treatment and lower level in P50 treatment. The crude lipid was in the range of 3.80 to 6.78%. The maximum total ash was found in P30 treatment and minimum level was

noticed in P0 treatment. The growth and biochemical composition of the *L. rohita* fed *P. stratiotes* is shown in Table (4). The growth response determined in terms of final body weight, weight gain, specific growth rate (SGR) and feed conversion ratio(FCR) of *L. rohita* showed significant difference ($P<0.01$) by different levels of *P. stratiotes* in their diet. The highest weight gain and specific growth rate were observed in P30 group and the lowest value was noticed in P50 group. The FCR was gradually decreased with increase in supplementation of *P. stratiotes* in the diet. The experimental animal consumes 30% diet elicited the lower value of FCR as compared to other diets.

With respect of body composition of *L. rohita* results showed that an increase in the *P. stratiotes* diets. Protein content was gradually increased and it was maximum in fish fed with 30% *P. stratiotes* diet. The lipid, ash and energy contents were also increased with increasing the *P. stratiotes* diets up to 30%. However, nitrogen free extract showed the opposite trend. Generally the highest protein, lipid ash and energy contents significantly ($P<0.001$) found at fish maintained at both of 20 and 30% *P. stratiotes* level in the diets with values of (29.06 ±0.03 ; 32.08 ± 0.009), (0.96 ±0.008 ; 1.16 ± 0.03), (20.24±0.02 ; 20.95 ± 0.02), (2.47±0.01 ; 2.62 ± 0.02) and nitrogen free extract (49.74± 0.05 ; 43.22 ± 0.10) Also *L. rohita* consumed 30% *P. stratiotes* diet produced the significant increment of protein (32.08 ± 0.009), lipid (1.16 ± 0.03), ash (20.95 ± 0.02), energy (2.62 ± 0.02) and nitrogen free extract (43.22 ± 0.10) respectively. Finally the lowest values were obtained with the fish maintained at 50% *P. stratiotes* diet with the values of (22.07 ± 0.02, 0.49 ± 0.03, 19.08 ± 0.012, 10 ± 0.0158 and 36 ± 0.002) respectively. The haematological parameters were affected significantly ($P<0.01$) by different levels of *P. stratiotes* in the diet (Table 5). The present result revealed that, the haematological parameters like RBC count and Hb% were high in 30% *P. stratiotes* diet and it drastically decreased beyond this level. The WBC count showed an increasing trend as the increase in inclusion of *P. stratiotes* meal in the diet and the maximum value was witnessed in P50 treatment.

The activity of the digestive enzymes is listed in Table 5. The amylase, protease and lipase activity were determined from foregut, midgut and hindgut regions of *L. rohita*. The amylase activity was higher in the foregut than midgut and hindgut. The secretion of protease was high in mid gut followed by hindgut and foregut. Lipase activity was highest in the hindgut followed by midgut and foregut. The results indicate that, fish fed 30% *P. stratiotes* diet elicited higher activity of digestive enzymes than other treatments.

4. Discussion

The results of the present study reveals that water lettuce *P. stratiotes* leaf meal can be incorporated in the diet of *L. rohita* up to 30% without any negative growth response. The diet containing the plant meals extends the diet acceptability and growth up to 30%. Above the optimum level of inclusion of plant proteins, retarded growth of fishes was observed. Most of the fish species tolerate the replacement or inclusion level of plant protein below 40-50%. Above this limit the growth retardation started due to low acceptance of feed, digestion related problems and effect of anti-nutritional factors on growth.

The present result is in line with the results of several studies conducted with the inclusion of different aquatic weeds in different fish species. The aquatic weeds such as *Ipomea reptans* and *Lemna minor* could be important sources of protein, vitamins and minerals suitable for incorporation in fish diet Kalita *et al.,* (2007) [18]. According to Sivani *et al.,* (2013) [34] found that anti nutritional factors were found to be present in *Nymphaea* weeds, their levels were within tolerable limits and consumption of these plants would not result in any deleterious effect on the growth of fish. The feeding fish with high levels of *Nymphaea* leaf meal (50% diet) has not yielded positive result, optimum levels of incorporation 40% yielded better results in terms of growth. Ray and Das (1992) [31] reported that growth performance of rohu fingerlings fed on composted water lettuce, *Salvinia cuculata* incorporated diets in laboratory conditions. The results indicated the possibilities of incorporation of composted *Salvinia* leaf meal in supplementary diets for the Indian major carp, substituting the conventional diet up to 20% level. Protein digestibility was highest (94.0%) from *Eichhornia crassipes*, followed by *Lemna polyrhiza* and *Nymphoides cristatum*. Digestibility of lipid from *Nymphoides cristatum* and *Lemna polyrhiza* was higher, whereas, digestibility of carbohydrates was found to be highest in *Eichhornia crassipes*. Similarly, Ray and Das (1995) [32] observed that the suitability of dried aquatic weed *P. stratiotes* leaf meal as a diet stuff in pelleted feed for *L. rohita* fingerlings and their results indicated the possibility of including *P. stratiotes* leaf meal in pelleted feed up to 45% level for the in Indian major carp.

Hematological parameters are routinely used for the evaluation of physiological environment and husbandry stressors in fishes (Rainza-Paiva *et al.,* 2000) [30]. Soivo and Oikari (1976) [35] stated that, the applications of haematological techniques have proved valuable for fishery biologist in assessing the health of the fish and monitoring stress response. According to Oyawoye and Ogunkunle (1998) [26] found that, haematological components of blood are valuable in monitoring feed toxicity, especially with feed constituents that affect the formation of blood. The present investigation reveals that, fish fed with P30 diet elicited increased level of RBC and WBC count and Hb (%) which may be due to the high amount of iron content present in *P. stratiotes* leaf. Similar to the present result, Bello Nuhu Ozovehe and G.C. Nzeh (2013) [4] found that, 10% *Moringa oleifera* leaf meal diet (Plant protein) enhanced the RBC count and Hb in African catfish *Clarias gariepinus*. Poston and Livingston (1969) [28], reported that lower haematocrit were observed in brook trout fry fed a diet containing a high level of vitamin E (5000 mg/kg). Similarly Baker and Davies (1996) [3], also reported that African catfish fed high a-tocopheryl acetate dose (500 mg/kg dry feed) were observed to have significantly lower hematocrit than fish fed the basal diet. According to Klinger *et al.,* (1996) [21] observed that haemoglobin and parked cell volume (PCV) have been suggested as tests that can be carried out on routine basis in fish hatchery as a check on fish health status. Enzyme activity has been reported in the gut of several fish species (Al-Tameemi *et al.,* 2010; Lazzari *et al*., 2010; Chaudhuri *et al*., 2012) [2, 22,8]. From the present studies, it is revealed that, the experimental animal fed with 30% *P. stratiotes* meal supported to increase the activity of digestive enzymes. Nandeesha *et al.,* (1998) [25] found that the higher levels of *Spirulina* (60-100%) supplementation reduced the intestinal protease and lipase in *Cyprinus carpio* and it supports the observations made in present study. Groundnut leaf and field beans meals increased the amylase activity in the foregut and midgut prawn head meal chicken intestine diets showed an

elevated amylase secretion in the foregut but decreased gradually in the mid and hindgut of *L. rohita* (Sethuramalingam and Haniffa, 2012) [33]. Herbivorous and omnivorous fishes tend to have high amylase activity in comparison to the carnivores, since the former need it to breakdown the polysaccharides that dominate their natural diets (Hidalgo *et al.,* 1999) [15].

5. Conclusion

In conclusion the study clearly revealed that replacement of fishmeal with 30% inclusion of *P. stratiotes* meal gives better overall performance of *L. rohita* compare with other diets. And as such substituting it with fish meal in fish diet showed great promise with potentially reduces the cost of fish feed. Although experiment concludes that, fishmeal could not replaced totally with plant; however partial replacement can be done by using *P. stratiotes* meal would optimum for the growth of *L. rohita*.

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Table 1: Proximate composition of *Pistia stratiotes* (g%)

Crude protein	15.96 ± 0.13
Crude lipid	5.10 ± 0.29
Total ash	22.20 ± 1.56
Fibre	11.08 ± 33
Nitrogen Free Extract (NFE)	45.66 ± 0.19

Table 2: Percentage composition (%) of the experimental diets

Ingredients (g/100g diet)	Experimental diets					
	P 0	P10	P20	P30	P40	P50
Fishmeal	36.5	31.5	26.5	21.5	16.5	11.5
Groundnut oil cake	36.5	31.5	26.5	21.5	16.5	11.5
Tapioca flour	13.5	13.5	13.5	13.5	13.5	13.5
Rice bran	13.0	13.0	13.0	13.0	13.0	13.0
Pistia meal	-	10	20	30	40	50
Vit & mins	0.5	0.5	0.5	0.5	0.5	0.5

Source: Hardy (1980)

Table 3: Proximate composition of experimental diets (g%)

Diets	Crude protein	Crude lipid	Total ash	NFE
P 0	32.12 ± 0.10	5.25 ± 1.05	12.25 ± 0.89	50.38 ± 0.45
P 10	32.80 ± 0.85	5.70 ± 2.10	13.01 ± 1.10	48.49 ± 0.12
P 20	33.72 ± 1.15	6.01 ± 1.01	13.50 ± 0.72	46.77 ± 0.34
P 30	35.44 ± 0.70	6.78 ± 0.85	14.56 ± 0.10	43.22 ± 0.10
P 40	30.17 ± 1.28	4.30 ± 1.28	13.10 ± 1.28	52.43 ± 0.09
P 50	25.85 ± 0.23	3.80 ± 0.12	10.72 ± 0.85	59.63 ± 0.05

Table 4: Growth and Biochemical parameters of *Labeo rohita* fingerlings feed with different level of *Pistia stratiotes* leaf meal diet for 80 days. Each value is the mean (X ± SD) standard deviation of triplicate groups of fish.

Parameters	P 0	P10	P20	P30	P40	P50
Initial wt (g)	1.23 ±0.008	1.23 ±0.008	1.23 ±0.008	1.23 ±0.008	1.23 ±0.008	1.23 ±0.008
Final wt (g)	2.03±0.06	2.25±0.002	2.37±0.05	2.75±0.12	2.13±0.10	1.86± 0.004
Weight gain (%)	0.71± 0.01	1 ±0.10	1.14± 0.13	1.55± 0.007	0.89±0.09	0.61 ±0.003
FCR	3.94 ± 0.03	3.80± 0.02	3.67±0.02	3.50 ±0.02	4.06±0.04	5.08 ±0.06
SGR (%)	0.88 ± 0.02	0.90 ± 001	0.94 ± 0.01	0.98 ± 0.02	0.81 ±0.09	0.69 ±0.09
Body composition of fish						
Crude protein	25.68 ±0.02	27.11±0.02	29.06 ±0.03	32.08±0.009	27.05 ±0.02	22.07±0.02
Crude lipid	0.52 ±0.02	0.67 ± 0.008	0.96 ±0.008	1.16 ±0.04	0.79±0.009	0.49 ±0.03
Total Ash	19.85±0.04	20.04±0.02	20.24±0.02	20.95±0.03	20.18±0.02	19.08±0.01
Energy	2.24±0.008	2.33±0.01	2.47±0.01	2.62±0.02	2.29±0.009	2.10±0.01
NFE	53.95± 0.01	52.18±0.007	49.74± 0.05	45.81± 0.04	51.98±0.009	58.36±0.002

Mean of three replicates ± SEM

Weight gain (%) = (Final wet weight – Initial wet weight) × 100

Feed conversion ratio = Feed consumption (mg) / Weight gain (mg)

Specific growth rate = $lnw_2 - lnw_1 / t \times 100$

NFE = 100 – (Crude protein+ Crude lipid +Crude fibre+ Toal Ash)

Table 5: Haematological parameters and digestive enzymes activity of *Labeo rohita* fingerlings feed with different level of *Pistia stratiotes* leaf meal diet for 80 days. Each value is the mean (X ± SD) standard deviation of triplicate groups of fish.

Parameters	Initial value of fish	Final value at different *Pistia* inclusion rates (%)					
		P 0	P10	P20	P30	P40	P50
RBC ($\times 10^6 mm^{-3}$)	1.05±0.04	1.68±0.01	1.90±0.8	2.12±0.2	2.38±0.08	1.81±0.01	1.49±0.008
WBC($\times 10^6 mm^{-3}$)	25.01±0.2	25.21±0.05	25.32±0.2	25.12±0.4	25.01±0.2	26.12±0.1	31.11±0.09
Hb(g%)	2.98±0.09	3.71±0.009	4.05 ±0.04	4.33± 0.02	4.50±0.04	4.02± 0.02	3.48± 0.01
Enzymes	**Amylase (µg maltose / mg of protein/hr)**						
Foregut	210±35	305±08	315±14	330±10	362±17	325±15	300.±10
Midgut	120±17	175±19	180±20	187±05	210±10	178.±12	173±14
Hindgut	50±12	88±71	96±10	103±15	143±08	90±14	83±44
Protease (µg tyrosine / mg of protein/hr)							
Foregut	133±5	183±14	190±18	195±13	212±14	195±15	173±10
Midgut	182±15	235±13	243±12	255±3	268±5	250±9	230±8
Hindgut	160±10	190±9	201±9	220±9	258±3	219±14	185±3
Lipase (µg lipase / mg of protein/hr)							
Foregut	80±07	110±11	123±10	130±15	158±09	125±16	105±15
Midgut	210±10	247±18	250±17	258±03	280±15	260±12	245±12
Hindgut	230±12	268±18	273±11	280±11	313±09	282±12	260±25

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