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Growth efficacy and Feed utilization of fresh water Fishes *Cirrhinus mrigala* (Ham.) and *Cyprinus carpio* L. fed with *Limonia acidissima* L.

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Abstract

The effect of dietary Limonia acidissima L. fruit (LF) on the growth and feed utilization was investigated in Cirrhinus mrigala and Cyprinus carpio. Mrigal fingerlings of about 5.30 ± 0.03 g and Carp fingerlings of about 3.50 ± 1.50 g were fed, diets supplemented with three concentrations (1.5 %, 3 %, and 6 %) of herbal diet for 30 and 60 days. Survival, specific growth rate, feed conversion rate, feed efficiency and relative growth rate parameters were significantly different ($p < 0.05$) and higher in experimental groups compared to control. The highest weight gain (17.75 ±0.19; 50.91 ±0.39) and feed conversion ratio (0.92 ±0.01; 0.64 ±0.03) of C. mrigala were observed at 30 and 60 days respectively in 3% herbal diet fed group and lowest in control. In addition, the highest weight gain (18.97 ±0.10; 51.53 ±0.20) and feed conversion ratio (0.56 ±0.007; 0.42 ±0.005) of C. carpio were observed in 3% herbal diet and lowest in control. Protein efficiency ratio and specific growth rate for C. mrigala and C. carpio respectively were higher in fishes fed with 3% herbal diet and lowest in control. The overall difference in parameters between C. mrigala and C. carpio were significant among experimental group than the control ($p < 0.05$). These results reveal that an underutilized fruit of medicinal plant Limonia acidissima L. enhance the growth and metabolic parameters of C. mrigala and C. carpio fingerlings.

Keywords: Feed utilization, *Limonia acidissima L.* fruit, growth performance, fresh water carps.

Introduction

Aquaculture has increased prominently in India during the past few decades. Mass cultivation of fishes for human consumption has led to intensive aquaculture. Handling, crowding, transport, grading, poor water quality and other factors have lead to stress emergence[1]. These conditions make the fishes susceptible to decreased growth efficiency and disease. Close quartered cultivation strategies have extensively reduced the growth efficacy and feed utilization rate of fishes. Moreover accidental abrasions that occur during such cultivation strategies cause the spread of infectious diseases. In India, intensive freshwater aqua culturing has been of great significance due to the economic loss caused by the insufficient growth and aquaculture diseases. Hence a promising method should be developed to improve the growth and the defense mechanism of fish. Dietary supplementation of natural products as *Eclipta alba*[2], *Zingiber officinale*[3], *Ocimum sanctum*[4], *Achyranthes aspera*[5,6], *Solanum trilobatum*[7], *Aloe vera*[8], *Astragalus radix* and *Scutellari radix*[9], have been known to improve the growth and immune parameters of the fishes against disease causing agents. *Limonia acidissima* (Rutaceae) fruit is a tropical plant species in the Indian subcontinent, commonly known as 'Vilampazham' in Tamilnadu, India. The tree is distributed widely in India and in few parts of the world[10, 11]. The fruits are woody and has been extensively used in Indian folk medicine, in ayurveda for the treatment of blood impurities, leucorrhoea and in yunani medicine as diuretic[12]. The fruits of L. acidissima (LF) contain flavanoids, phytosterols, glycosides, saponins, tannins, coumarins, triterpenoids, carbohydrates, vitamins, amino acids as its chemical constituents[13-15] and tyramine derivatives have also been isolated. The fruit also has the antioxidant property[16]. Anti-oxidant activities and the traditional applications of the *L. acidissima* fruits possessing wound healing property were demonstrated by Choudhary et.al.[17]. The present investigation aims to evaluate the dietary effect of LF powder on weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) on fresh water fishes *Cyprinus carpio* and *Cirrhinus mrigala*.

Materials and Methods

Experimental animal and conditions: Common carp *Cyprinus carpio* and *Mrigal Cirrhinus mrigala* fingerlings (average weight 3.50 ± 1.50 g and 5.30 ± 0.03 g, respectively) were procured from Fish seed farm, Induced carp spawning centre, Tamil Nadu Fisheries Development Corporation Limited (TNFDC) at Aliyar (Tamil Nadu, South India). The health status of the fishes was observed immediately after arrival. All fishes were given 10% formalin wash and were maintained as two separate batches with 3 sets (each set containing 4 tanks) of 200 L fiber reinforced plastic tanks. Fishes were maintained under natural photo period (12h dark/12h light) and suitable

temperature for 35° ± 2° C. Water was changed every 4 days to maintain quality and sufficient aeration was provided with suitable aerators. They were acclimatized for 15 days with a balanced fish diet.

Preparation of experimental herbal diet: The fruits of *Limonia acidissima* L. (LF) were collected from Vellingiri hills (Tamil Nadu, India). The fruit was taxonomically identified and authenticated (Voucher No. BSI/SC/5/23/09-10/Tech-319.) by Dr. P. Sathayanarayana, Deputy Director, Botanical Survey of India, Southern Circle, Tamilnadu Agricultural University (Govt. of India), Coimbatore, Tamilnadu, India. Fruits were cleaned; fruit pulp was scooped, shade dried and pulverized using electrical blender powdered. The powdered fruit pulp was sieved using 50 µm sized mesh sieve and kept in a dry, clean, airtight jar before being added to diet. Balanced basal diet without fruit supplement was used as control feed. The experimental diet was prepared by supplementing the basal diet with different concentrations (15 g, 30 g, and 60 g/kg of basal feed) of LF (table 1). All the dry ingredients for each of the experimental diets were added and mixed thoroughly with deionized water (250 ml/kg dry ingredients mixture) for 15 min. The resulting dough was pelletized, air dried at room temperature for 48 h and then stored in dry, airtight containers until fed.

Table-1
Diet supplements and their composition

Ingredients	Control diet (g/Kg)	Experimental diet (g/Kg)
Rice bran	450	450
Groundnut oil cake	200	200
Maize	100	100
Finger millet	100	100
Pearl millet	100	100
Agrimin super forte*! (Vitamin and minerals pre mixture)	50	50
Limonia acidissima fruit (15, 30, 60 g/kg)	Absent	Present
Proximae composition of feed (%)		
Moisture	6.14	
Crude protein	38.76	
Crude lipid	11.42	
Ash	9.68	
Fibre	3.26	

***** Nutritional value per kg : Vitamin A - 700000 IU; Vitamin D_3 - 140000 IU; Vitamin E - 500 mg; Vitamin B12 - 1000 mcg; Folic Acid - 100 mg; Nicotinamide - 1000 mg; Copper - 1200 mg; Cobalt - 150 mg; Iron - 1500 mg; Zinc - 3000 mg; Iodine - 325 mg; Selenium - 10 mg; Magnesium - 6000 mg; Manganese - 1500 mg; Potassium - 100 mg; Calcium - 270 gm; Phosphorus - 130 gm; Sulphur - 7.2 gm; Fluorine - 300 mg.

Experimental design and feeding frequency: C. carpio (n=600) and C. mrigala (n=600) fingerlings were divided into to two separate batches A and B respectively. Each batch contained three sets with each set having four groups namely control, group I, group II, and group III (each group contains 50 fishes). Control group fishes were fed with normal balanced diet, Group I fishes were fed with 1.5% LF mixed diet, group II fishes were fed with 3% LF mixed diet, and group III fishes received 6% LF mixed diets for 30 and 60 days (in triplicates). After experimental feeding trial, 25 fishes from each group were randomly selected and were subjected to growth parameter analysis. The growth performance, including percentage weight gain (RGR), specific growth rate (SGR), feed conversion ratio (FCR), Protein efficiency ratio (PER) for each group was determined, as described by[18].

"Relative growth rate (RGR)=" ("Final body weight-initial body weight")"÷Initial weight ×100"
Specific growth rate (SGR)=(Ln Final weight-Ln Initial weight)÷No of days in trial×100
Feed conversion ratio (FCR)=Feed given (dry wt)÷Weight gain (wet weight)
Protein efficiency ratio (PER)=wet weight gain by fish (g)÷Protein intake (g)

Statistical analysis: The data were expressed as mean ± standard error (SE). Statistical analysis of data involved, one-way analysis of variance (ANOVA) followed by Tukey's pair wise comparison test using SPSS (version 16 for windows). Differences were considered statistically significant when $p < 0.05$.

Results and Discussion

Results: Growth response and feed utilization efficiency of C. mrigala.

Growth analysis of C. mrigala fed with experimental diets for 30 and 60 days are shown in table 2 and table 3 respectively. All fish fed actively and appeared healthy. Survival of the fish during the experiment ranged from 91.3 % to 95.3%. There were significant growth responses of fish fed with Limonia acidissima fruit (LF) meal over the control. The best growth response was achieved at 30 g/kg of LF supplemented feed while a moderate growth was observed in the fish fed with control diet (table 2 and 3). However, significant difference was observed in the growth performance across the different LF concentrations ($p < 0.05$). The growth rate was significantly lower in group I and group III when compared to group II. There was a significant increase in the feed conversion ratio (FCR), relative growth rate (RGR), specific growth rate (SGR), Feed efficiency (FE) and Protein efficiency ratio (PER) of fish fed with LF meal over the control group ($p < 0.05$). Highest FCR and PER value was observed in those fish groups fed with 30 g/kg of experimental feed.

Growth response and feed utilization efficiency of C. carpio: Fish fed LF diet showed significantly ($p < 0.05$) higher weight gain over the control. Growth parameters such as feed conversion

ratio (FCR), relative growth rate (RGR), specific growth rate (SGR), Feed efficiency (FE) and Protein efficiency ratio (PER) of fish fed with LF meal over the control group ($p < 0.05$). Highest weight gain, SGR and FCR was observed in those fish groups fed with LF diet at 30 g/kg feed (Table 4 and 5).

Table-2
Mean growth performance and feed utilization of *C. mrigala* fingerlings fed experimental diets containing *Limonia acidissima* fruit (LF) for 30 days

Parameter*	Control	LF (1.5%)	LF (3%)	LF (6%)
IBW (g)	5.36 ±0.015^{a}	5.38 ±0.014^{ab}	5.41 ±0.011^{b}	5.41 ±0.01^{b}
FBW (g)	19.01 ±0.26^{a}	19.40 ±0.17^{a}	23.16 ±0.20^{c}	21.76 ±0.21^{b}
LWG (g)	13.65 ±0.26^{a}	14.01 ±0.17^{a}	17.75 ±0.19^{c}	16.35 ±0.20^{b}
SGR (%d-1)	1.83 ±0.20^{a}	1.85 ±0.01^{a}	2.1 ±0.01^{c}	2.01 ±0.01^{b}
RGR %	254.84 ±4.9^{a}	260.45 ±3.40^{a}	328.05 ±3.70^{c}	302.18 ±3.75^{b}
FCR	1.19 ±0.02^{c}	1.16 ±0.01^{c}	0.92 ±0.01^{a}	0.99 ±0.01^{b}
FE	0.85 ±0.02^{a}	0.87 ±0.01^{a}	1.10 ±0.01^{c}	1.01 ±0.01^{b}
PER	0.15 ±0.03^{a}	0.23 ±0.04^{a}	0.31 ±0.06^{c}	0.26 ±0.05^{b}
Survival rate (%)	99.3 ±0.02^{c}	92.67 ±0.02^{a}	96.67 ±0.02^{b}	91.33 ±0.02^{a}

The values are expressed as mean ± standard errors of mean. Means in a given row with different superscript letters were significantly different at $p < 0.05$. * IBW= Initial body weight, FBW = Final body weight, LWG = Live weight gain, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed conversion ratio, FE= Feed efficiency ratio, PER = Protein efficiency ratio.

Table-3
Mean growth performance and feed utilization of *C. mrigala* fingerlings fed experimental diets containing *Limonia acidissima* fruit (LF) for 60 days

Parameter*	Control	LF (1.5%)	LF (3%)	LF (6%)
IBW(g)	5.36 ±0.02^{a}	5.38 ±0.01^{ab}	5.41 ±0.01^{b}	5.41 ±0.01^{b}
FBW(g)	46.60 ±0.21^{a}	48.80 ±0.35^{b}	56.32 ±0.39^{d}	53.52 ±0.4^{c}
LWG(g)	41.22 ±0.21^{a}	43.41 ±0.35^{b}	50.91 ±0.39^{d}	48.12 ±0.41^{c}
SGR (% d-1)	1.62 ±0.31^{a}	1.6 ±0.32^{b}	1.75 ±0.34^{d}	1.66 ±0.33^{c}
RGR %	769.54 ±4.54^{a}	806.82 ±6.92^{b}	940.95 ±7.43^{d}	889.30 ±7.51^{c}
FCR	0.78 ±0.00^{d}	0.74 ±0.05^{c}	0.64 ±0.03^{a}	0.67 ±0.03^{b}
FE	1.28 ±0.01^{a}	1.34 ±0.01^{b}	1.57 ±0.01^{d}	1.48 ±0.01^{c}
PER	3.28 ±0.02^{a}	3.46 ±0.03^{b}	4.05 ±0.03^{d}	3.83 ±0.03^{c}
Survival rate (%)	100 ±0.0^{c}	93.3 ±0.16^{b}	95.3 ±0.16^{b}	91.3 ±0.16^{a}

The values are expressed as mean ± standard errors of mean. Means in a given row with different superscript letters were significantly different at $p < 0.05$. * IBW= Initial body weight, FBW = Final body weight, LWG = Live weight gain, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed conversion ratio, FE= Feed efficiency ratio, PER = Protein efficiency ratio.

Table-4
Mean growth performance and feed utilization of *C. carpio* fingerlings fed experimental diets containing *Limonia acidissima* fruit (LF) for 30 days

Parameter*	Control	LF (1.5%)	LF (3%)	LF (6%)
IBW (g)	3.62 ±0.03^{a}	3.56 ±0.03^{a}	3.58 ±0.04^{a}	3.64 ±0.03^{a}
FBW (g)	13.56 ±0.05^{a}	14.57 ±0.08^{b}	22.55 ±0.12^{d}	16.83 ±0.17^{c}
LWG (g)	9.94 ±0.06^{a}	11.01 ±0.09^{b}	18.97 ±0.10^{d}	13.19 ±0.17^{c}
SGR (%d-1)	1.91 ±0.01^{a}	2.04 ±0.02^{b}	2.67 ±0.016^{d}	2.22 ±0.018^{c}
RGR %	275.3 ±3.6^{a}	310.7 ±5.3^{b}	531.9 ±7.3^{d}	363.4 ±5.9^{c}
FCR	1.09 ±0.014^{d}	0.97 ±0.017^{c}	0.56 ±0.007^{a}	0.83 ±0.014^{b}
FE	0.92 ±0.011^{a}	1.03 ±0.017^{b}	1.77 ±0.024^{d}	1.21 ±0.020^{c}
PER	2.37 ±0.03^{a}	2.67 ±0.04^{b}	4.57 ±0.06^{d}	3.12 ±0.05^{c}
Survival rate (%)	100 ±0.0^{c}	95.3 ±0.16^{b}	98.6 ±1.6^{b}	95.3 ±1.02^{a}

Values are expressed as mean ± standard errors of mean. Means in a given row with different superscript letters were significantly different at $p < 0.05$. *IBW= Initial body weight, FBW = Final body weight, LWG = Live weight gain, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed conversion ratio, FE= Feed efficiency ratio, PER = Protein efficiency ratio.

Table 5
Mean growth performance and feed utilization of *C. carpio* fingerlings fed experimental diets containing *Limonia acidissima* fruit (LF) for 60 days

Parameter*	Control	LF (1.5%)	LF (3%)	LF (6%)
IBW(g)	3.62 ±0.03[a]	3.56 ±0.04[ab]	3.58 ±0.04[b]	3.64 ±0.03[b]
FBW(g)	37.12 ±0.10[a]	39.14 ±0.16[b]	55.10 ±0.21[d]	43.66 ±0.34[c]
LWG(g)	33.50 ±0.12[a]	35.58 ±0.17[b]	51.53 ±0.20[d]	40.08 ±0.36[c]
SGR (% d-1)	1.69 ±0.01[a]	1.74 ±0.01[b]	1.98 ±0.01[d]	1.81 ±0.01[c]
RGR %	927.42 ±9.21[a]	1003.51 ±13.49[b]	1444.43 ±17.72[d]	1125.13 ±20.19[c]
FCR	0.65 ±0.01[d]	0.60 ±0.01[c]	0.42 ±0.005[a]	0.55 ±0.01[b]
FE	1.55 ±0.02[a]	1.67 ±0.02[b]	2.41 ±0.03[d]	1.84 ±0.02[c]
PER	3.98 ±0.014[a]	4.30 ±0.021[b]	6.19 ±0.025[d]	4.73 ±0.043[c]
Survival rate (%)	100 ±0.0[c]	93.3 ±0.16[b]	95.3 ±0.16[b]	91.3 ±0.16[a]

The values are expressed as mean ± standard errors of mean. Means in a given row with different superscript letters were significantly different at $p < 0.05$. *IBW= Initial body weight, FBW = Final body weight, LWG = Live weight gain, SGR = Specific growth rate, RGR = Relative growth rate, FCR = Feed conversion ratio, FE= Feed efficiency ratio, PER = Protein efficiency ratio

Discussion: The competence of the immunostimulants in promoting the growth rate was well known. Recent experiments have revealed that many substances can be used to enhance the growth and non-specific immunity of the fish with special reference to their route of administration. The results suggest that dietary supplementation of Limonia acidissima fruit (LF) promoted the growth of Cirrhinus mrigala and Cyprinus carpio fingerlings and enhances nutrient utilization, which is evident from the improved weight gain, FCR, FE, PER, SGR and RGR after 30 and 60 days feeding trial. The feed conversion ratio values obtained in all experimental groups were significantly ($p < 0.05$) higher when compared to control (table 2-5). These results also emphasize that diets supplemented with 3% (group II) LF meal showed significant ($p < 0.05$) increase in all the growth parameters when compared to 1.5% (group I) and 6% (group III) at 30 and 60 days time period. However the growth parameters of group III were lower when compared to group II which elucidates that supplementation of LF at higher concentration require more time for conversion. It is observed that the FCR of 3% experimental diet fed group is higher at 60 days than at 30 days time period which indicates that the FCR values increases with increase in feeding time.

Medicinal herb when given as dietary supplement promoted the growth and feed conversion efficiency in shrimp[19]. Red clover Trifolium pretense was found to be a good growth enhancing agent for tilapia O. aureus. Faster growth on juvenile pike perch (Sander luciopera) fed with medicinal plants was observed when compared to the control diet[20]. According to Kim et al.[21] the unknown factors in various medicinal herbs led to the increased growth in experimental groups. The presence of flavonoid in the Limonia acidissima fruit might indicate the stimulation of growth in fishes[22]. However, bioflavonoids in plants have estrogenic activity, and studies have shown that estrogen promotes growth in common carp[23]. Results of medicinal plants showing promising enhancement of growth were reported in different fishes such as common carp Cyprinus carpio[24], guppy Poecilia reticulata[25], the cichlid Cryptoheros nigrofasciatus[26], and red sea bream Pagrus major[27]. Therefore, LF diet that promotes growth in C. mrigala and C. carpio should be tested for its efficacy to induce efficient and economical propagation in other fishes. The best feed utilization values observed in diets supplemented with LF meal suggested that the addition of LF meal to the basal diet might be reliable.

Conclusion

In conclusion, this study provides the significance of Limonia acidissima L. fruit mixed diet upon increasing the growth parameters of Cirrhinus mrigala and Cyprinus carpio. The results show the promising potential role of this fruit and its use in promoting the growth of fresh water fishes thereby making it an significant method to achieve sustainable, economical, and safe fish production. To our knowledge this is the first report showing the efficacy of the underutilized fruit of Limonia acidissima as herbal diet supplement to fish feed. Further analysis on hematological, immunological, and molecular aspects would elucidate more information on the significance of the fruit as fish feed.

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