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Growth performance of *Labeo rohita* (Hamilton) yearlings through use of fermented *Ipomoea aquatica* leaf meal as fish feed

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Abstract
A study was carried out to evaluate the fermented *Ipomoea aquatic* leaf meal as fish feed of Indian major carp yearling, *Labeo rohita* for a period of 90 days. Four different diets were prepared with fermented *Ipomoea aquatica* viz. 0%, 30%, 40% and 50% inclusion level and fed to rohu fingerlings in treatments T-I, T-II, T-III and T-IV respectively. Unfermented *Ipomoea aquatica* was taken as a reference diet. A total of 84 nos. of fingerlings were introduced in 12 nos. of rectangular cement cistern with size (2.7m x 1.0m x 1.5m) at a density of 7 nos. per tanks with three replicates. The initial average weight of fishes were 6.12 ± 0.23 g, 6.09 ± 0.16 g, 6.33 ± 0.26 g and 6.03 ± 0.66 g in T-I, T-II, T-III and T-IV respectively. Fishes showed significantly ($p<0.05$) higher net weight gain, SGR, FCR, PER & survivability and production in treatment group T-III, where fishes fed with diet containing 40% fermented *Ipomoea aquatica*. Though there was no significance ($p<0.05$) difference observed between T-III & T-II in relation to SGR, FCR, PER and was also found to be similar where fishes fed with 30% fermented *Ipomoea aquatica.* Lowest net weight gain, SGR, FCR, PER and survivability was recorded in group T-IV where fish fed with diet containing 50% *Ipomoea aquatica.* However, the growth of fish in the control group (T-I) was almost comparable to T-IV treatment group. The water quality parameters were not affected by the level of different diets and found to be desirable range for fish growth. These findings indicated that diet containing 40% & 30% fermented *Ipomoea aquatica* appears to be sufficient for obtaining optimum growth in Indian major carp yearling, *Labeo rohita* in cement cisterns.

Keywords: Indian major carp, yearling, fermented, water hyacinth

1. Introduction

Supplementary feeding is an important aspect to fulfill the nutritional requirements of farmed fishes in aquaculture system as natural production alone may not support the complete or balanced nutrition to promote growth of farmed fishes. In farm made feed, aquatic and terrestrial macrophytes have been used as non-conventional feed stuff (NCPF). These NCPF usually contain some anti-nutritional factors which effect the growth of fish and limit its utilization [15]. Plant proteins are less expensive than animal protein but contain anti-nutritional factor that limit its application in animal feed [34]. Various authors have suggested incorporation of NCPF like *Ipomoea* in fish feed [7, 26]. Leaves from aquatic plants – floating and submerged such *as Lemna sp, Azolla sp, Cerataphyllum demersum, Hydrilla and Verticillata* [39] *and Salvinia cuculata, Trapa natans, Lemna minor* and *Ipomoea reptans* have been reported as fish feed by [1]. *Ipomoea aquatica* which is commonly known as water spinach, Morning glory, Forsake, Water bind weed, Chinese water-spinach is a common emergent aquatic plant belonging to the family *Convolvulaceae* that can grow freely over the water surface or over marshy ground. It is native to Central south China and widely cultivated in China, Indonesia, Thailand, Vietnam, Myanmar, Philippines, Bangladesh and in India [23]. [37] analyzed the composition of dried leaf and stem powder of *Ipomoea aquatica* and reported 24-34% crude protein, 2.7-3.9% lipid, 13% ash and 10.2-12.7% crude fibre. Although this plant is use as human food by the inhabitant of the area where it grows, less information is available on its nutritional value.

However, it was observed that in the current form of processing method NCPF can't be utilized at higher level without compromising growth and production [39]. Therefore, alternative measures are looked for to increase its nutritional value. Enhancement of nutritive values for NCPF can be achieved by fermentation or ensilation [39].

These action not only provides a more digestible product but it may also increase the protein content from the microbial biomass along with secretion of digestive enzymes particularly amylase and protease. Based on water activity, only fungi and yeast were termed as suitable micro-organisms for fermentation process. It was thought that due to high water activity requirement, bacterial cultures might not be suitable for fermentation process [25]. *Aspergillus* is the most popular species due to its capacity to produce enzymes such as hemicellulases, hydrolases, pectinases, protease, amylase, lipases and tannases [27, 20]. Fungal fermentations result in degradation of various anti-nutritional factors, an increase in amount of small-sized peptides and improved content of both essential and non-essential amino acids [22]. Fungi-based fermentation by several species of *Aspergillus* genus have been used to ferment SBM by *A. oryzea* [10, 19], *A. niger* [20]. Fungal fermentation using *A. oryzae* eliminated TI (trypsin inhibitor) from 2.6 mg/g to zero as reported by [10, 19]. Fermentation with *Aspergillus* almost completely eliminates phytate, protease inhibitors and flatulence factors resulting in a protein source for feed with highly available phosphorus [14, 29]. According to [34], fermentation with *A. oryzae* did not affect the essential amino acid concentrations but increased the concentrations of glycine, glutamine and aspartic acid. Fermentation of cereals and their bland with legumes is a potentially important processing method which improves the nutritive value such as availability of protein and amino acid profile [35].

2. Material and Methods

2.1 Experimental site and design

The experiment was performed using Completely Randomized Design (CRD) in 12 numbers of rectangular cement cisterns of size (2.7m x 1.0m x 1.5m) provided with 6 inches soil bed. Cisterns were initially dried, cleaned, treated with Quick lime (CaO) @ of 250 kg/ha and covered with nylon net to prevent escape of fish. Water depth was maintained at 1.2 m ± 5 cm during the entire experimental period.

2.2 Stocking of Fish and growth studies:

A total of 84 nos. of *Labeo rohita* yearlings were randomly introduced into the experimental tanks at a density of 7 nos. per tank (2 nos/m³) and each group were provided with three replicates. The growth parameters were calculated according to the formula given by [28].

2.3 Fermentation and Feed formulation:

Colleted *Ipomoea aquatica's* stem and leafs were rinsed of dirt, sundried and ground into fine powder using grinder. The grind powders were sieved to obtain a homogeneous particle size and fermented by Solid State Fermentation method [18].
For fermentation, ATCC culture of *Aspergillus oryzae* was inoculate in Potato Dextrose Agar (PDA) media and kept in a incubator at 30˚C for 120 hours for the development of spores [5]. These spores were transferred in 250 ml Erlenmeyer flasks that containing 20 g of *Ipomoea aquatica*. The substrate was moistened to 50% moisture with tap water, autoclaved at 121°C for 25 min, and cooled at room temperature. Then the substrate was inoculated with 0.06 g of spores and incubated at 30°C for 120 hr. Contents of the harvested flasks were thoroughly mixed with a glass rod before sampling for various analyses and also incorporated in feed ingredients. Other dietary ingredients used include MOC, wheat flour, rice polish and vitamin mineral mixture. Four iso-proteinaceous (25%) diets were prepared viz. control diet (T-I) containing unfermented dried *Ipomoea* stem & leaf powder and test diet T-II, T-III & T-IV where fermented *Ipomoea* stem & leafs were included at 30%, 40%, 50% respectively. The% of different ingredients use for preparation of diets is given in Table-1. Wheat flour and vitamin mineral mixture were kept constant in all the four diets. The required amount of dried ground ingredients was weighed carefully for different diets and mixed with required amount of water for 15-20 minutes allowed to stand & dough were obtained. The dough was then transferred to an aluminium container and cooked/steamed for half an hour in a pressure cooker. After cooled, the calculated proportion of the vitamins and minerals mixture were incorporated and mixed well. The dough then pressed through a pelletizer machine to get uniform sized pellets which were spread on a tray and dried in an oven at 60°C ± 5°C for overnight. Feeding was done by simply broadcasting @ 3% body weight once in a day during morning hour between 7.30 - 9 am. The feed ration was adjusted fortnightly.

2.4 Estimation of proximate composition:

Proximate analysis of ingredients and prepared diets for moisture, dry matter, ash content, crude protein, crude fibre, crude fat and nitrogen free extract were determined following [1].

2.5 Water quality monitoring:

For analysis of different water quality parameters samples were collected from all tanks in the morning 6:00 - 09:00 – hours at 15 days interval and analyzed by following the standard methods [2].

2.6 Statistical analysis

The data obtained from experimental groups were subjected to one-way Analysis of variance with Statistical Package for Social Sciences (SPSS version 16.0 for windows, 2013) to determine the level of significance.

3. Results and Discussion

The ingredients and their percentage used to prepare diets is given in Table 1.

Table 1: Proximate compositions of feed ingredients on dry matter basis.

Ingredients	Dry Matter (%)	Protein (%)	Lipid (%)	Fibre (%)	Moisture (%)	Ash (%)	NFE (%)
Ipomoea aquatica	85.59 ± 0.37	27.42 ± 0.80	1.97 ± 0.17	11.62 ± 0.23	14.41 ± 0.79	13.47 ± 0.46	32.11 ± 1.21
Fermented *Ipomoea aquatica*	83.09 ± 0.58	30.31 ± 0.58	1.78 ± 0.12	9.67 ± 0.06	16.91 ± 0.25	11.56 ± 0.09	29.77 ± 0.41
MOC	88.76 ± 0.01	31.50 ± 0.11	8.07 ± 0.05	12.77 ± 0.11	11.24 ± 0.02	6.78 ± 0.05	29.64 ± 0.10
Rice polish	89.85 ± 0.05	12.75 ± 0.02	8.22 ± 0.10	23.16 ± 0.01	10.15 ± 0.05	16.84 ± 0.03	28.88 ± 0.10
Wheat flour	89.84 ± 0.02	12.80 ± 0.01	1.78 ± 0.12	1.36 ± 0.02	10.16 ± 0.02	1.11 ± 0.10	72.79 ± 0.02

*Values of each parameter are mean ± SE of triplicate determinations

Table 2: Proximate composition of experimental test diets on dry matter basis

Test Diet	Dry Matter (%)	Protein (%)	Lipid (%)	Fibre (%)	Moisture (%)	Ash (%)	NFE (%)
T-I	87.32 ± 0.58	24.64 ± 0.75	4.19 ± 0.12	9.99 ± 0.52	12.68 ± 0.12	12.31 ± 0.16	36.19 ± 0.13
T-II	80.88 ± 0.43	24.44 ± 0.54	6.51 ± 0.58	5.56 ± 0.58	19.20 ± 0.58	16.81 ± 0.58	27.48 ± 0.58
T-III	80.29 ± 0.58	24.90 ± 0.66	5.24 ± 0.15	6.5 ± 0.46	19.71 ± 0.46	15.30 ± 0.91	28.35 ± 1.04
T-IV	79.44 ± 0.69	24.98 ± 0.12	4.98 ± 0.77	7.5 ± 0.23	20.56 ± 0.80	16.46 ± 0.49	25.52 ± 0.23

*Values are mean±SE of triplicate determinations

3.1 Proximate composition of the ingredients & experimental diets:

Proximate composition of the ingredients and the experimental diets were summarized in Table 1 & 2. The present findings in relation to nutrient levels of *Ipomoea aquatica* are similar to that reported by [26, 37]. The present investigation revealed differences in protein levels between fermented and unfermented *Ipomoea aquactica* leaf meal and showed about 2.89% increase in protein levels in fermented than compared to unfermented *Ipomoea aquatica*. [17] also reported 2.64% increase in protein levels in *Ipomoea aquatic* after fermentation. Similarly increase of protein levels in fermented macrophytes and legumes have also been reported by [24, 4].

3.2 Growth performance of fish

Present investigation showed significantly ($p<0.05$) highest net weight gain, specific growth rate, food conversion ratio, protein efficiency ratio, survivability and production of fishes in T-III, where fishes fed with diet containing 40% fermented *Ipomoea aquatic*. Similarly better growth was also observed in T-II, where the fishes were fed with 30% fermented *Ipomoea aquatic* than compared to T-I and T-IV. The better performance of fishes in T- III and T-II may be due to better acceptability and reduction of anti – nutritional factor in *Ipomoea aquatic* leaf meal after fermentation, which otherwise may present in the unfermented non-conventional feed stuff. Further, the nutritional quality of *Ipomoea aquatica* in terms of protein is increases by the process of fermentation. The fiber content of raw *Ipomoea aquatica* was also reduced after the fermentation process [12]. reported that solid state fermentation with *S. cerevisiae* decrease the anti-nutritional factor and improve the nutritional value of soyabean meal. Better utilization of fermented *Lemna* leaf meal in diets for rohu compared to raw was also recorded by [3]. The improved acceptability and biological value due to fermentation also been observed by [31]. The improved nutritional value and considerable decrease in anti- nutritional factor due to fermentation and thereby enhancement in fish growth was reported by several authors [38, 30].

The highest growth rate was observed in T-III, where 40% fermented *Ipomoea aquatica* was included is probably due to better utilization by *Labeo rohita* fingerlings at that level. [30] observed similar results in rohu fingerlings fed diet containing 40% seaseam seed meal fermented with *Lactobacillus acidophilus*. Fish fed diet containing 40% fermented grass feed showed better weight gain, specific growth rate and protein efficiency ratio [29].

The lowest growth performance and survivability was recorded in T-IV, where fishes fed with diet containing 50% fermented *Ipomoea aquatica*. The inferior growth rohu fingerlings in T-IV may be due to non-utilization of fermented *Ipomoea aquatica* at higher levels. It is may be due to the fact that major part of weight gain is related to the deposition of protein accretion is a balance between protein anabolism and catabolism. Further gastric empting rate or solubility of the protein has been shown to affect the utilization of dietary protein [6, 9]. Furthermore the higher inclusion of fermented *Ipomeoea aquatica* (50%) compromise the growth performance due to presence of microbial endogenous non digestible components, lesser nutrient contents due to increase of bulk density and high fiber content. It is reflected by the lower FCR (1.29 ±. 06) and PER (1.59 ± 0.18) value observed in T-IV. [11] observed late acceptability of water hyacinth incorporated diet at 75% inclusion level by rohu fry. However, the growth of fishes in T-IV was almost comparable to T-I treatment where unfermented Ipomoea aquatic was included.

3.3 Specific Growth Rate (SGR)

The specific growth rate was found to be significantly ($p <0.05$) higher in T-III (3.23 ± 0.16) compared to T-I and T-IV, though it was not significantly ($p <0.05$) varied with T-II. Similarly, [29] also reported better SGR in the fish fed diet contain 40% fermented grass pea.

3.4 Feed utilization parameter

Considerably a better food conversion ratio was recorded in T-III (1.42 ± 0.06) and found to be significantly ($p <0.05$) different from T-I, T-II and T-IV. The 40% fermented *Ipomoea aquatica* inclusion level improved acceptability and biological value of the diet [31, 8] have reported improved outcomes of FCR between 1.2 & 1.5. The improved nutritional value and considerable decrease in anti- nutritional factor due to fermentation and thereby enhancement in fish growth has also been reported. Furthermore, the nutritional quality of *Ipomoea aquatica* in terms of protein is increases by the process of fermentation.

Improved protein efficiency ratio was recorded in T-III (2.23 ± 0.024) and significantly ($p <0.05$) higher compared to T-I and T-II. Though it was not significantly ($p <0.05$) varied with T-II. The better protein utilization was observed in T-III and T-II (2.06 ± 0.23) which reflected on the growth of the fishes. It is may be due to improvement of nutritional quality of *Ipomoea aquatica* in terms of protein increases by the process of fermentation and showed about 2.89% increase in protein levels in *Ipomoea aquatic* after fermentation.

3.5 Survivability

The average survival rate was recorded in T-III (90.47) followed by T-I (85.71), T-II (80.95) and T-IV (76.19). The mortality recorded in all the treatment was due to abnormal high temperature in between 60th & 75th day of rearing.

3.6 Water quality parameters

The water quality parameters were recorded in desirable range during the experimental period and no significant ($p < 0.05$) difference was observed among the treatments.

During the experimental period DO values were ranges between 3.0 - 7.8 mgl^{-1} which was within the tolerance limit of carps. Dissolved oxygen (DO) content of water plays a significant role in the metabolic process of fishes. The quantity of feed supplied also influencing the demands for oxygen [13, 33, 16] reported that supplementary feeds are among the input factors which determines the oxygen balance oxygen balance of ponds.

Higher alkalinity (110- 334 mgl^{-1}) and hardness (110-375 mgl^{-1}) were observed during investigation which indicating of productive water. As in the experiment no inputs other than lime was added and further productivity of water has no relevance to the present finding, However the values obtained were within the productive range.

Nitrite is an intermediate product of the aerobic nitrification bacterial process, produced by the autotrophic *Nitrosomonas* bacteria combining oxygen and ammonia. A lower nitrite concentration (0.1 - 0.4 mgl^{-1}) during the study which were within acceptable limits. The ideal and normal measurement of nitrite is zero in any aquatic system. [32] recommended that nitrite concentration in water should not exceed 0.5 mgl^{-1}.

Nitrate is harmless and is produced by the autotrophic *Nitrobacter* bacteria combining oxygen and nitrite. Nitrate is considered as not lethal for aquaculture practice. Nitrite on further decomposition in the presence of *Nitrobacter* gets converted to nitrate, which is harmless. According to [36] nitrate is relatively nontoxic to fish and not cause any health hazard except at exceedingly high levels (above 90 mg/l). In the present study the nitrate level ranged between 0.3 - 2.7 mgl^{-1}. [32] described the favourable range of nitrate to be 0.1 mg/l to 4.0 mg/l for fish culture.

4. Conclusion

From the present findings it is now apparently established that *Labeo rohita* can utilize fermented dietary *Ipomoea aquatica* at the level of 30% and 40% inclusion level in the formulated diet but higher inclusion level (50%) results in inferior growth. Higher inclusion of fermented *Ipomeoea aquatica* (50%) compromise growth performance of *Labeo rohita* due to presence of microbial endogenous non digestible components, lesser nutrient contents due to increase of bulk density and high fiber content. However, the inclusion of 40% fermented dietary *Ipomoea aquatica* showed superior growth and survival among the treatments group. Therefore present studies indicated that 40% inclusion level of fermented *Ipomoea aquatica* not only reduce the waste input in the holding water but also results in higher growth rate. Moreover, present study reveals that fermented *Ipomoea aquatica* have potential growth promoting effect in *Labeo rohita* fingerlings. Furthermore, future study must be carried out using same amount of raw *Ipomoea aquatica* over fermented *Ipomoea aquatica* for proper validation evaluating the enzymatic activity, action of anti-nutritional factors etc.

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