Formulation of artificial feeds for Indian Carp (*Catla catla*) fry using aquatic plants (*Ipomea aquatica* and *Hydrilla vercillata*)

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Abstract- There is no recommended low cost feed available from fry to fingerling stage of Indian carps in Sri Lanka. Hence, this study was aimed on utilizing locally available aquatic plants *Ipomea aquatica* and *Hydrilla vercillata* and discarded dry fishmeal in feeds of *Catla catla* fry to cut down the feed cost. The experiment was conducted in a complete randomized design. Three experimental feeds were formulated using different protein sources while keeping other ingredients constant (T1= 1:1 discarded dried fish meal: *Hydrilla vercillata*, T2= 1:1 discarded dried fish meal: *Ipomea aquatica*, T3 = discarded dried fishmeal) and a commercial feed was used as the control. *Catla catla* fry were stocked at a density of 75 fry per m^2 in 12 pond units. Fry were fed at the rate of 5% of the body weight. Growth and water quality parameters were measured weekly and feeding behavior was also observed. Data were analyzed using the one way Analysis of Variance (ANOVA) procedure in SAS. All treatment feeds were accepted by fish and palatability was same. *Hydrilla vercillata* incorporated feed was moderately stable in water whereas, T2, T3 and commercial feeds were comparatively stable. Total body length, wet body weight and specific growth rate (SGR) of the fry were significantly different ($p<0.05$) and the highest SGR were recorded in fry fed with T3. Fish showed isometric growth against all the feeds. The highest FCR was recorded with the fry fed with aquatic plants incorporated feed. Survival rate of fry and water quality parameters were not significantly affected by the treatment feeds ($p>0.05$). The lowest feed costs were recorded in T1 and T2 feeds and it was 60% less compared to commercial feed. In conclusion, there is a potential benefits of using discarded dried fishmeal, *Ipomea aquatica* and *Hydrilla vercillata* in aqua feeds for fry stage of *Catla catla*.

Index Terms- Aqua feed, *Catla catla*, Discarded dried fishmeal, *Hydrilla vercillata*, *Ipomea aquatica*

I. Introduction

In Sri Lanka, inland fisheries sector contributes around 0.2% to the Gross Domestic Production (GDP), showing 70% growth of inland fishing contribution. Further, it accounts for about 20% of the total fish production of the country. The sector provides direct and indirect employments to considerable number of people. Moreover, fish products are an important source of animal protein and around 70% of Sri Lankans consume fish and related products to fulfill their protein requirement [1].

Inland fish production mainly consists of capture based fishing activities and is mainly dependent on exotic species such as Tilapia spp, Indian carps and Chinese carps [2]. However, sustainability of aquaculture production depends on proper feeding and farm management [3]. Feeding of fish has become one of the critical management practices today, as it occupies 50-60% from the total cost of production. Therefore, it is important to produce low cost feeds for small scale farmers in order to reduce the cost of farm operation. In Sri Lanka, there is no recommended low cost feed available from fry to fingerling stage of Indian carps. Some farmers have been using their own ingredients in formulating fish feed, without considering availability of nutrient in the raw materials and the nutritional requirement of the fish. Thus, those feeds do not contain required amount of nutrients and it is impossible to achieve higher growth rate of fry under local conditions.

Of the feed ingredients, protein source is one of the expensive ingredients in the formulated feed. Fish meal is still an essential ingredient in the diets and it is also an expensive feed ingredient compared to other protein sources and thus represents a significant cost element in feed and production cost. This has necessitated the search of alternative sources available locally in the country. In this context, use of certain potential aquatic weeds offer excellent scope as the nutrient laden materials are naturally grown in the entire country without much agronomic care. Many aquatic plants such as *Eichornia crassipas, Hydrilla verticillata, Salvinia aculata, Ipomea aquatica, Pistia* spp. etc. contain fairly high amount of protein. Aquatic plants also contain high amount of vitamin E, vitamin C and mineral elements required for normal growth and development of fish [4]. *Ipomea aquatica* and *Hydrilla verticilata* are currently used in many South East Asian countries as the alternative feed ingredients. Those two aquatic plants have fairly high feeding value, with moderately high protein content [5]. The intensive growth of aquatic weeds in culture ponds and reservoirs are one of the prevailing problems in fish production. If aquatic weeds are used effectively in feed formulation, it will be a solution for management of aquatic weeds. Thus, present study was undertaken to formulate cost effective feeds for the fry to fingerling stage of *Catla catla*, using locally available feed ingredients including aquatic plants under a semi intensive culture system.

II. METHODOLOGY

Location

The study was conducted at the National Aquaculture Development Center, Ibbankaduwa, Dambulla, which is located in DL_1 agro ecological zone.

Preparation of Mud Ponds

Four rectangular mud ponds, each with a size of 20 x 20 x 1.5 m^3 were used for the experiment. Each pond was divided into three sub units with a size of 10.5 x 20 m^2 using two fry nets (mesh size = 4 mm) and separated units were considered as the replicates. Pond preparation was done, two weeks prior to the stocking of fingerlings and followed disinfection, fertilization and water management.

Calcium hypochlorite (at a rate of 30 g/m^2) was used as the disinfecting agent and was applied manually on the floor of water drained dry ponds, which remained for about two weeks. Cow dung culture prepared using 500 g/m^2 fresh cow dung, 4 g/m^2 urea and 4 g/m^2 triple super phosphate (TSP), was used to fertilize the ponds. In preparation, cow dung was thoroughly mixed with water in an aluminum tough with a volume of 100 m^3 to make a fine textured mixture. Then, dissolved urea and TSP solution were added to the culture and mixed well. The final mixture was allowed to settle for four days and equal amount of prepared mixtures were added manually to each pond unit. Each pond was filled up to 0.3 m height with water from Ibbankaduwa tank after fertilization. After seven days of fertilization, water level was increased up to 1.22 m height and a secchi disk reading was maintained around 30 cm. When the secchi disk reading was lower than the 30 cm, water was flushed out through outlet and new water was pumped to the pond. Cow dung culture (250 g/m^2 cow dung, 2 g/m^2 urea and 2 g/m^2 TSP) was mixed the water.

Fish Seed and Stocking

Fourteen days old, *Catla catla* fry (mean body length = 2.2 cm and mean body weight = 0.1 g) were used as the fish seed (n = 15) in each replicate. After one week of fertilization of ponds, the fry were stocked at a stocking density of 75 fry per m^2 in each pond unit. Required number of fry for stocking were measured using counting cups.

Experimental Feeds

Three experimental feeds (T1, T2, T3) were formulated by trial and error method considering protein, essential fatty acids, fiber and energy requirements of the fish (Table 01). Commercial feed (a tropical commercial fish feed; No 00) was used as the control treatment. Discarded dried fish meal, *Ipomea aquatica*, *Hydrilla vercillata* were cut into small pieces and allowed separately for sun drying, to reduce the moisture content approximately up to 20% for two days. Soya bean was dried under the sun for 48 hrs. in order to reduce the trypsin inhibitors. Coconut poonac was also sun dried for about 4 hrs. After drying, ingredients were grounded separately to make a powder, and sieved using a sieve (mesh size = 1 mm). Finally, ground samples were packed separately in polythene bags until feed preparation.

The required amounts of feed ingredients for the experimental diets T1, T2 and T3 were measured and macro ingredients were mixed thoroughly. Then, vitamin, mineral pre-mixture and coconut oil were added gradually to the dry ingredients mixture and mixed thoroughly. Wheat flour was used as the binding agent and warm water (60 C^0) was added into the mixture and feed balls were prepared manually (Plate 01).

Table 01: Composition of treatment diets

Ingredients (%)	Treatment feeds		
	T1	**T2**	**T3**
Discarded dried fish meal	30	30	60
Ipomea aquatica meal	30	-	-
Hydrilla vercillata meal	-	30	-
Soya bean	13	13	13
Rice bran	12	12	12
Coconut poonac	8	8	8
Wheat flour	5	5	5
Coconut oil	1	1	1
Vitamin pre mix	0.5	0.5	0.5
Mineral pre mix	0.5	0.5	0.5

T1 T2 T3 Commercial

Plate 01: Different types of experimental feeds

Feeding

Feeding trays (polythene sheets attached to a polyvinyl chloride pipe frames) with a size of 1 m x 1 m^2 were used to feed the fry. Two feeding trays were placed at the two corners of each pond unit. Four wooden poles were used to place the feeding tray inside the water and the four corners of the feeding tray were bonded to the wooden poles (Plate 02).

Plate 02: Placement of feeding tray

The required feed quantity for subsequent period was calculated after measuring the total wet body weight and fry were fed at the rate of 5% of the body weight (per day), three times at 8.00 a.m., 12.00 noon and 5.00 p.m.

Data Collection and Calculations

The feeding behavior of fish, palatability, acceptance and stability of feeds in water were determined by daily observations. The nutritional quality of all experimental feeds were determined by proximate analysis following the standard AOAC [6] procedure. Total body length and wet body weight of the fry were measured using a ruler and electronic analytical balance respectively in weekly intervals, taking random sample (n=15) from each replicate. The specific growth rate (SGR) was calculated at the end of the each week using following equation.

$$SGR = \frac{[\ln(mean\ final\ wet\ weight) - \ln(mean\ initial\ wet\ weight)]}{Number\ of\ days} x\,100$$

Feed conversion ratio (FCR) was calculated by standard equation at the end of the experiment.

Four water samples from each replicate were collected at around 5.00 am - 7.00 am to measure the water quality parameters weekly. Temperature was measured using a thermometer at about 30 cm depth of water and pH, dissolved oxygen (DO), hardness, NH_3 concentration were measured using a Hach kit (model FF-1A). Light penetration was measured using a secchi disc. Water sample was collected from each replicate, close to each feeding trays in order to measure the NH_3 concentration. Cost of feed ingredients were noted and cost analyses were done for each feed to find out the least cost aqua feed for *Catla catla* fry.

Fish Harvesting

At the end of the experiment, the total fish of each replicate was harvested separately using fry harvesting net with mesh size of 2 mm and counting was done using a counting cup. The survival rate was calculated using following equation.

$$Survival\ rate\ (\%) = \frac{Number\ of\ fish\ survived}{Number\ of\ fish\ stocked} x\,100$$

Data Analysis

Continues variables were analyzed using Analysis of Variance (ANOVA) procedure of Statistical Software for Data Analysis (SAS, ver. 9.0) to evaluate the best incorporation level of feed. Mean separation was done by Tukey's Studentized Range Test (TSRT) and statistical significance was declared at $p < 0.05$.

The following experimental model was applied to analyze the data.

$X_{ij} = \mu + T_i + E_{ij}$

where:

X_{ij} = Any observation made in the experiment

M = Observed mean

T_i = Effect of experimental diet (i = T1, T2, T3 and commercial feed)

E_{ij} = Residual error

Relationship between body length and body weight were found by MINITAB computer software using following equation,

$$\ln w = b \ln l + \ln a$$

whereas,

w = weight in gram, l = length in centimeter, a and b = constants

III. RESULTS AND DISCUSSION

Proximate composition of Experimental Feeds

Crude protein content of the experimental feeds ranged from 27.5 - 42.0% while commercial feed had the highest crude protein content (Table 02). Crude fat and fiber content of feeds

were more or less similar and the highest gross energy was recorded in T2 feed where incorporation of 1:1 discarded dried fish meal and *Ipomea aquatica*. This may be due to the presence of high amount of carbohydrate (38.6%) in *Ipomea aquatica* [5]. Gross energy content of other two test diets and control also changed within a narrow range.

Table 02: Nutrient composition of formulated feeds

Proximate composition	Treatment feeds			
	T1	T2	T3	Commercial*
Moisture, %	8.1	8.9	9.1	-
Ash, %	25.6	17.6	20.5	12.0
Crud protein, %	27.5	30.4	36.3	42.0
Crude fat, %	9.1	9.6	9.9	10.0
Crude fiber, %	5.9	6.6	4.6	4.0
Crude energy, Kcal/ kg	3289	3843	3473	3350

*Values were provided by the manufacturer

Ash content of the test feeds was much higher than the commercial feed and the highest ash percentage was observed in T2 feed.

Quality of the Formulated Feeds

As the fry were not familiar with tray feeding, they did not accept the feeds at once. However, after 2 - 3 days later, they were familiar for tray feeding and observed that, they crowded around the trays while feeding. During the 1st week of the experiment, T1, T2 and T3 feeds were not readily accepted by fry. However, commercial feed was readily accepted as they were fed with commercial feed before the experimental period. However, after the 1st week, the fry soon became accustomed to the experimental feeds and observed that, they fed actively throughout the experimental period. The acceptance of feed depends upon variety of factors, such as availability, appearance, particle size and organoleptic properties relating to smell, taste and texture [7]. Commercial feed usually contains high quality fish meal and has a comparatively good smell, which attracts fry to the feed. This may be also a reason for readily acceptance of commercial feeds than other formulated feeds.

From the beginning of the experiment, commercial feed was highly palatable to young fish. Texture of commercial feed was soft than the other formulated feeds. Soft feed often proving to be more palatable to the fish [8, 9] and fish may be capable of handling larger feed particles when they are soft [10]. During the 1st week, palatability of T1, T2 and T3 feeds were moderate. However, after the 1st week, most of the fry readily fed on all other feeds as commercial feed. Palatability of T1, T2 and T3 feeds may be due to fish meal or other cumulative effect of combination of several feed ingredients. These results further confirmed that, the replacement of fish meal by 50% *Ipomea aquatica* or *Hydrilla vercillata* leaf meal did not affect the palatability of feeds.

T2, T3, and commercial feeds were highly stable in water, where the T1 feed was moderately stable. Jayaram and Shetty [11] indicated the fat content in feed is known to give the compactness for the feed by preventing the entry of water. The higher fat content of T2, T3 and commercial feeds may be the reason for the high stability in water.

Growth Parameters and Cost of Feeds

The wet body weights and length gained by fish were significantly different ($p<0.05$) among treatments (Table 03). At the end of the study period, mean wet body weight and length achieved by young fish varied between 2.70 g - 4.82 g and 5.77 – 6.68 cm respectively. The mean wet body weight and length were similar between the fry fed with T1 and T2 feeds and fry fed with T3 and commercial feed ($p>0.05$). However, body weight and length were significantly greater ($p<0.05$) in the fry fed with T3 and commercial feed compared to the feeds incorporated with aquatic plants. Degani, Ren-Zvi [12] established a positive relationship between growth rate and protein content of diet. The present study also showed a significantly higher growth with high protein levels in commercial and T3 feed than the low protein levels in T2 and T1.

Table 03: Effect of treatment feeds on growth performances

Variable	T1	T2	T3	Commercial	SE
Wet body weight (g)					
Day 0	0.1	0.1	0.1	0.1	0.01
Day 7	0.51^{a}	0.57^{b}	0.69^{b}	0.89^{c}	0.08
Day 14	0.70^{a}	0.80^{a}	1.58^{b}	1.33^{b}	0.22

Day 21	1.20[a]	1.36[a]	2.13[b]	2.01[b]	0.23
Day 28	2.70[a]	2.94[a]	4.82[b]	4.22[b]	0.50
Total body length (cm)					
Day 0	2.2	2.2	2.2	2.2	0.08
Day 7	3.60[a]	3.73[a]	4.01[b]	4.08[b]	0.11
Day 14	3.92[a]	3.92[a]	4.84[b]	4.68[b]	0.24
Day 21	4.70[a]	4.76[a]	5.37[b]	5.23[b]	0.17
Day 28	5.77[a]	5.99[a]	6.68[b]	6.47[b]	0.21
SGR	10.95[a]	11.14[a]	12.66[b]	11.96[c]	0.94
FCR	0.98[a]	0.95[a]	0.67[b]	0.71[b]	0.14
Survival rate (%)	73.73	74.12	75.12	75.34	4.15
Feed cost (Rs.)*	82.15	79.90	124.40	181.90	Total

[a, b, c] values with different superscripts within each row differ significantly $p < 0.05$
*In Sri Lankan Rupees and did not statistically analyzed.

This slight reduction of body weight and length gain of young fish fed with 42% protein level in commercial feed compared to T3 may due to exceeding of optimum protein requirement of fry of *Catla Catla*. Kalla, Bhatnagar [4] indicated that, the optimum protein requirement of *Catla catla* was about 40% the supplementary feeds. Further, [13] also concluded that, daily growth rate of *Catla catla* was higher in feeds with 30 and 40% protein.

Further, higher growth rate observed in the study may be also due to the tray feeding (trays were placed at the middle layer of the water) as *Catla catla* is a middle layer feeder. Therefore, fry could have consumed feed freely without wasting energy to find the feeds. Further, as Khan, Ahmed [14] stated, feeding 5.0 - 5.5% of body weight per day could achieve optimum growth and efficient feed utilization for carps. Hence, properly balanced supplemental feeds with reliable feeding rate may be helpful to enhance the growth of *Catla catla* fry. Composition of feed and the size of the ration are the most important factors affecting growth. The higher growth observed for fish fed with the experimental feeds may be due to different ingredients used in different proportion.

The regression coefficient (b) of four feeds was closed to 03 which indicated, the fish in all treatments showed an isometric growth. It also indicated that, the fish fed with treatment feeds showed a proportional growth with their length and weight (Figures 01 - 04). The reliable b value of near to the 3 may be attributed to nutrient composition of feeds and better ecological factors.

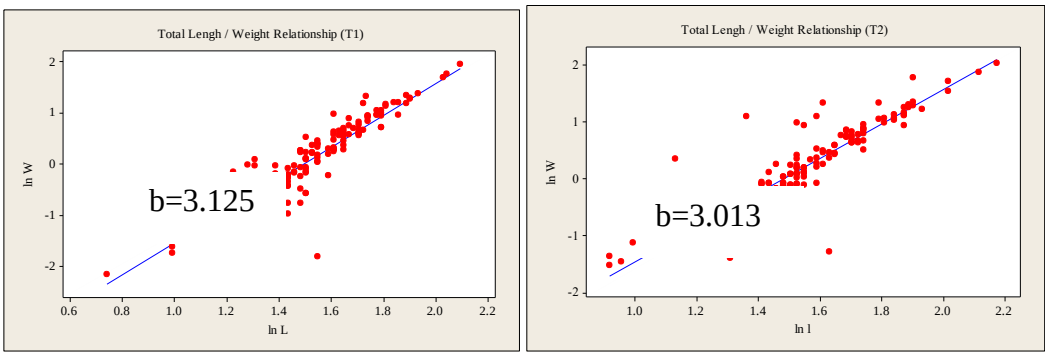

Figure 01 and 02: Total length weight relationship with T1 and T2 feeds

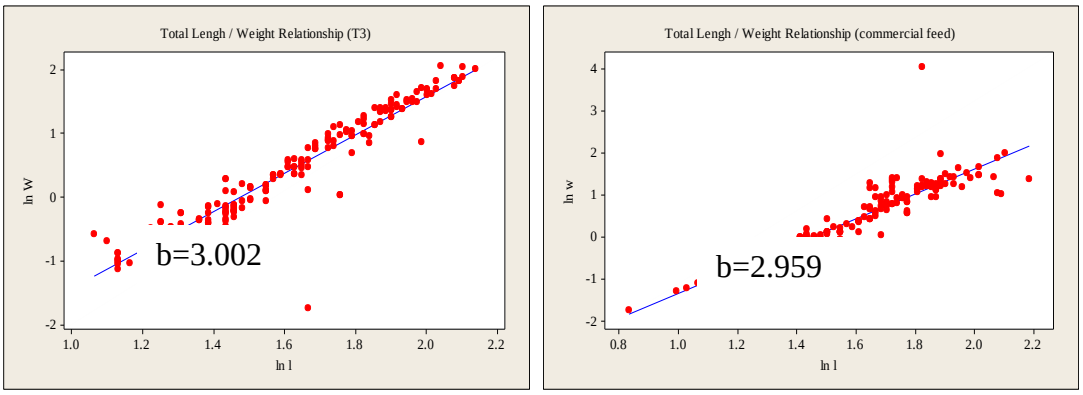

Figure 03 and 04: Total length weight relationship with T3 and commercial feeds

Specific growth rate of fry fed with experimental feeds was significantly different ($p<0.05$) and the highest was reported with T3. It was lower ($p<0.5$) in fry fed with aquatic plants in their feeds compared to the fry fed with commercial feed. Further, SGR increased linearly up to the 36.3% protein level and there after it declined. As Maqbool [15] reported, the SGR values of *Cyprinus carpio specularis* increases almost linearly with increasing dietary protein content up to 40% and then reaches a plateau. They further concluded that, highest SGR observed for fish fed with the 40% protein diet. The reduction of SGR values after the 36.3% protein in present study may be due to exceeding of optimum protein requirement of *Catla catla* fry.

Feed Conversion Ratio of fry fed with experimental feeds varied between 0.67 - 0.98, while FCR of T3 feed and commercial feed were significantly lower ($p<0.05$) compared to the aquatic plants incorporated feeds (Table 03). The results indicated that, a larger quantity of T1 and T2 feeds required for a unit weight gain of fry whereas, T3 and T4 feeds were required in lower quantity. Reduction of FCR value from low protein to high protein incorporated feeds also found by Kalla, Bhatnagar [4]. They invaded that FCR values were high for poor protein incorporated feeds and lowest for protein rich diets.

Survival Rate

Survival rate of the fry varied from 73.7 - 75.3% among the experimental feeds though the values were not significantly differ (p>005, Table 03). However numerically poor survival rate was found in fish fed on feeds with less protein whereas, higher value with increased protein content in feeds. The results were in accordance with those of Millamena [16], who observed the survival rate increases with increase of protein content in the fish feeds. According to Kalla, Bhatnagar [4], Indian major carps obtain 87.4% - 95.1% survival rate under field conditions. However, the survival rate that obtained in present study was lower than the past studies even in the field condition. The reduction of survival rate of present study may be attributed to the stress due to frequent handling and predatory problems such as dragonfly nymphs, cormorants.

Water Quality Parameters

Water quality parameters were not significantly changed by different treatments ($p>0.05$). Water temperature of ponds ranged from 26.5 ^{0}C - 29^{0}C during the study period and the water temperature variation of experiment was within the acceptable range (20^{0}C - 28^{0}C) recommended by Alabaster and Lloyd [17]. Though the pH of water did not differ significantly with the treatment feed, the highest mean value was recorded in the pond fed with commercial feed (9.0) and lowest value recorded with T1 feed (7.5). The acceptable limit of pH for the freshwater fish was 6.5 - 9.0 [18] and pH was within the favorable range in the present study. The results indicated that, pH of water increased with the increase of protein content in feeds and it agreed with the finding of Kalla, Bhatnagar [4]. Feeds with high protein release higher amount of ammonia than the feeds with lower protein and causes increase in pH due to formation of NH_4^+ complex in water [18].

According to Boyd [18], ammonia concentration between 0.6 and 2.0 mg/L are toxic to many fish species. During the present experimental period, the ammonia level were found to be low than toxic level in all treatments. The ammonia content in the ponds which were fed with T3 and commercial feed were slightly higher than that of feeds which were incorporated with lower proportion of fish meal (T1 and T2). It was in accordance with the results obtained by Parameshwaran, Edirisinghe [19], which showed that, when fish fed with fish meal based formulated feed, causes a slight increment of ammonia. However, the ammonia level in the present study was within the favorable limits as the highest value recorded was 0.47 mg/L.

Dissolved oxygen levels ranged from 6 - 11 mg/L with the treatment feeds. This range was above to the lethal level of 3.0 mg/L [18]. Numerically the highest and lowest mean values were recorded in the ponds which were fed with T1 and commercial feeds respectively and it showed that DO of pond water decreased with increased protein content of feeds. According to Kalla, Bhatnagar [4] the feeds with highest protein content have lower DO value than the feeds with lower protein content. Variation of secchi disk value which was the indication of transparency of water in the pond water ranged between 40 - 25 cm which indicated that natural feeds were available throughout the experiment period at reliable level for the fish growth. Hardness of pond water was not significantly different ($p>0.05$). It varied from 153 - 220 mg/L and was within the favorable range for freshwater fish since hardness values less than 20 mg/L causes stress and >300 mg/L is lethal to fish [20].

Cost Analysis of Formulated Feeds

The cost of formulated feeds showed a remarkable cost reduction with decrease in fish meal content in the test feeds (Table 03). Feed T1, in which 50% of fish meal was replaced by *Hydrilla vercillata* leaf meal seems to be the cheapest among all the feeds, as there is no commercial value for *Hydrilla vercillata* plant at present. *Ipomea aquatica* incorporated feed also could be considered as low cost feed (T2) than the feed prepared by locally available ingredient (T3). The cost for *Ipomea aquatica* incorporated feed depends on the market value, since it is used as a vegetable. However, the cost will be similar to that of *Hydrilla vercillata* incorporated feed when it is freely available. Both aquatic plants could be obtained free from the fish farms, especially in North Central province of Sri Lanka. Small scale farmers can involve their family members to collect the aquatic plants and for further processing. However, the preparation of *Ipomea aquatica* incorporated feed may be a tedious process for large scale farmers, as it contains only immature leaves. However, it will not a problem in small scale production and this should be decided on merits of individual farms with respect to their size, availability of plants, labour etc.

For small scale farmers, the formulated feed T3 which included locally available ingredients have an added advantage over commercial feed due to their better growth performance and cost, in a situation where, low cost ingredients are readily available. Cost for the feed T3 was about 50% less than from the cost of the commercial feed. Since feed cost occupied about 50 - 60% of the operation cost in the semi-intensive carp farming [21], T3 feed is the most cost-effective and affordable feed for the fish farmers compared to commercial feed.

IV. CONCLUSIONS

Aquatic plants could be incorporated as a non-conventional source of protein in the feed of *Catla catla* and discarded dried fish meal can also be used as an alternative source of protein for the high cost fish meal. The feed with aquatic plants and locally available ingredients have a good acceptance and palatability. Any of the formulated feeds T1, T2 and T3 does not affect the water quality of the pond. Fry exhibit an isometric growth for all formulated feeds and can obtain well balanced grown fingerlings at the end. Cost of the feed could be cut down up to 70% by using locally available ingredients for feed preparation and this cost can be further cut down by incorporating aquatic plants

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