EXECUTIVE SUMMARY

of the work done on

MAJOR RESEARCH PROJECT

"Standardization of Culture Technology of Duckweed (*Lemna* spp.) and its Utilization as Feed in Carp Poly-Culture System"

(Ref. No.: F. No. 41-70/2012 - SR)

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Supplementary feed is one of the key input in fish culture for elevating production, constituting more than 60 % of the input cost. Considering the ever increasing cost of conventional feed ingredients (rice bran, oiled seed cakes, fish meal etc.) and competition with other livestock for the same, it is vital to develop cost effective feeds by substituting the costly conventional fish feed ingredients with some cost effective locally available nutrient rich non-conventional feed resources (NCFR). Among various NCFR, aquatic plants constitute an important resource owing to high nutritive value and digestibility. In the category of aquatic plants, duckweeds has been proved to be the most promising due to their superior nutritive value and exceptionally fast growth rate. Duckweeds are small (1-15 cm) free floating aquatic plants with worldwide distribution. They belong to family Lamnacae and are widely available under five genera i.e. *Lemna, Spirodela, Landolita, Wolffi a*and *Wolfiella* having 37 species (FAO, 2009). Among these four genera, *Lemna* is the largest group of family Lamnaceae and *L. minor* is found to be the most promising one. Growth rate of duckweeds is faster than any other higher plant and more closely resemble the exponential growth of unicellular algae. Biomass of duckweeds get doubled in 16 hrs to 2 days under ideal conditions of nutrient availability (NH3-N = 7-12 mg/l, PO4-P = 4-8 mg/l), temperature (15 - 300C), pH (6.5 to 8.0) and sunlight. Nutrient content in duckweeds vary with the conditions in which they grow and crude protein in duckweeds has been found to vary between 15 - 45 % on dry matter (DM) basis. Duckweeds also have better array of essential amino acids than major plant proteins and more closely resembles animal protein. Further, its amino acid spectrum with regard to lysine (7.5 % of total protein) and methionine (2.6 % of total protein) is much higher as compared to other commonly used plant feed resources, except soybean. Duckweeds also have high levels of vitamin A and pigments particularly beta-carotene and xanthophylls. Duckweeds contain 92-94 % of moisture and harvested biomass can be easily sundried within a period of 24-48 hrs during dry hot summer months. All the duckweeds can be grown on naturally occurring nutrient enriched water (sewage effluents, domestic waste etc.) or manured water, with an average annual DM yield of 10-20 tonnes/ha.

Because of exceptionally fast multiplication rate and excellent nutritional profile of duckweeds, a number of studies have been carried out to produce and exploit duckweed biomass (fresh/dried) as livestock feed, including fish in laboratory or field conditions. Being an aquatic species, duckweeds have ample scope of application in aquaculture nutrition. Hence, it is vital to standardize technologies for production of protein rich duckweed biomass for utilization as feed or feed ingredient for fish, under region specific conditions.

In view of the above discussion, the project was undertaken with the aim to standardize the culture technology for two important duckweed species i.e. *Lemna minor* and *Lemna gibba* under local climatic conditions of Punjab and its utilization as one of the feed ingredient in semi-intensive carp polyculture system.

The research work under the project was taken up to fulfil the approved objectives by carrying out the studies on duckweed culture and its utilization as one of the feed ingredient for fish

I – Collection and maintenance of stock of *L. minor* and *L. gibba*

Two duckweed species (*L. minor* and *L. gibba*) were collected from different natural/manmade aquatic resources (wetlands, village ponds and road side ditches) from different districts of the State for maintaining culture stocks for experimental purpose. Stock of *L. minor* and *L. gibba* was maintained in poly sheet lined earthen pits (6 m2 of 0.3 m depth). Harvested duckweed biomass was sun dried and stored for experimental purpose

II. Standardization of culture technology of *L. minor* and *L. gibba* to develop package and practice of duckweed culture

For developing culture technology for duckweed (*L. minor* and *L. gibba*) culture, experiment was carried out for one year (Feb. 2013 to Jan. 2014) in 70 litre capacity plastic tubs (surface area – 0.228 m2), with 24 treatments [four manure/fertilizers – cow dung (CD), poultry droppings (PD), Cow dung : poultry droppings (CD:PD) and Urea+TSP at six different doses] for both the species (T1 to T24 for *L. minor* & T25 to T48 for *L. gibba*). Each tub was filled with 50 litres of water after providing a 2 cm soil layer. In each treatment, selected organic manure/inorganic fertilizer was added to provide the required nutrients for growth of duckweed. After 1 week of manuring/fertilization, duckweed was inoculated in all the treatments @ 75g (which covered half of the water surface). Post stocking manuring/fertilization was done at weekly intervals to provide the required nutrients to support sustained growth of duckweeds. Duckweed was allowed to grow in each treatment till it covered the whole water surface

To estimate the productivity and nutritive value of duckweeds, half of the duckweed biomass was harvested from each treatment every time it covered the whole water surface. The harvested biomass (wet) from all the treatments was weighed and sundried. For each treatment, all the harvestings of one month were pooled and stored separately in airtight poly bags for nutritive value (proximate composition) estimation.

Number of harvestings of *L. minor*

- ✓ During the period of one year, highest number of harvestings (30) was recorded in case of PD treatments followed by CD:PD (29), CD (28) and urea+TSP (16) treatments.
- ✓ Maximum 5 no. of harvestings were recorded in the month of March in all the PD treatments T7-T12.
- ✓ Overall results however revealed higher ***L. minor*** biomass production, in terms of no. of harvestings, during the months of March, May, July, August & September
- ✓ Although growth of ***L. minor*** declined with onset of winters, but the stock survived under low temperature conditions
- ✓ Inorganic fertilizers (urea+ TSP) could not support optimum duckweed growth as compared to organic manures (CD, PD & CD+ PD)

No. of harvestings of *L. gibba*

- ✓ During the culture period of one year, highest number of harvestings (17) was recorded in PD treatment followed by CD:PD (16), CD (14) and Urea+TSP (13) treatments.
- ✓ Maximum 5 no. of harvestings were recorded in the month of March in all the PD & CD + PD (1:1) treatments T31-T36 & T37-T42
- ✓ Overall results however revealed higher ***L.gibba*** biomass production, in terms of no. of harvestings, during the months of February, March & July
- ✓ However, *L. gibba* did not grow well in any of the treatments during the post monsoon months and all the stock vanished completely with the onset of winters

Duckweed biomass production

Out of four manures tried at six different levels, Poultry droppings (PD) was found to be most suitable manure in terms of maximum productivity of both duckweed species viz. *L. minor* (158 t/ha/yr) and *L. gibba* (81.70 t/ha/yr). However, out of two duckweed species, *L. minor* performed better than *L. gibba* in terms of production with all the manures.

Hence, out of two duckweed species, *L. minor* found to be more suitable w. r. t. growth response and number of harvestings under local climatic conditions throughout the year.

III. Nutritive value analysis of duckweeds, *L. minor* and *L. gibba*

Monthly harvested duckweed samples were pooled (weighted) and analysed for nutritive value (proximate composition estimation) from different treatments (*L. minor* – T1-T24, *L. gibba* - T25- T48) to know the most suitable manure and its appropriate dose for maximum duckweed biomass harvest along with superior nutritive value. Proximate composition was analysed with respect to crude protein, crude fat (ether extract), crude fibre and ash as per standard methods of AOAC (2000).

Nutritive value of duckweeds

***L. minor* -** Among all manures, throughout the culture period, CD:PD@ 600 kg/ha/wk resulted in significantly higher crude protein (26.27%). Further, monsoon season was found most suitable to harvest protein rich (CP-26.65 %) biomass with CD:PD.

***L. gibba* -** Among all manures, throughout the culture period, CD:PD @ 600 kg/ha/wk resulted in significantly higher crude protein (28.29%). However, PD resulted in maximum crude protein (33.97%) content in *L. gibba* during winter season (February)

- Results of the duckweed culture experiments showed that **suitable duckweed species** w. r. t. growth response under culture conditions throughout the year, no. of harvestings, biomass productivity, winter tolerance and nutritive value is ***L. minor***
- ***L. minor*** culture can be taken up under local climatic conditions of Punjab by using organic manures like p**oultry droppings** (@ 600 kg/ha/wk) and combination (1:1) of **cow dung and poultry droppings** (@ 600kg/ha/wk) during **pre-monsoon and monsoon months**.

IV. Formulating cost effective nutritionally balanced *Lemna* incorporated diets for carps

Two experimental studies were carried out to work out the efficacy of *Lemna* incorporated diets for carps fry and fingerlings

Experimental study 1.Efficacy of *L. minor* and *L. gibba* incorporated supplementary diets in carp fingerlings

Six month experimental study was conducted in FRP pools (5'3" x 3'8" x 2'5") to assess the efficacy of sun dried duckweeds (*L. minor* &*L. gibba*) in the diet of common carp, *Cyprinus carpio* fingerlings for 180 days. Ten duckweed based experimental diets were prepared by incorporating *Lemna* powder of both the species @ 10, 20, 30, 40 and 50 % (*L. minor* - D1 to D5, *L. gibba* –D6-D10) respectively by replacing basal (control) diet (C). Each pool was stocked with common carp fingerlings (Av. total body length 8.10 – 8.51cm**,** Av. body weight 8.25 – 8.50 g) @ 10/pool. Fish in each treatment was fed once a day during morning hours @ 2 % FBW. During the experimental period, water quality parameters, fish survival and growth was determined at regular intervals.

Water quality: Optimum water quality is required for optimum growth of fish under controlled conditions. In the experimental present study, the water temperature (14.03-14.35°C), pH (7.88-8.00), dissolved oxygen (4.77-5.67 mgl^{-1}), total alkalinity (419-436 mgl^{-1}), total hardness (406-445 mgl^{-1}), orthophosphate (0.76-1.69 mgl^{-1}) and ammonical nitrogen (0.02-0.10 mgl^{-1}) were well within the recommended range in all the treatments for supporting optimum growth in carps throughout the culture period and the differences among treatments were insignificant ($p<0.05$).

Fish Survival: At the termination of the experiment, 100 % survival of *C. Carpio* was recorded in all the treatments and control showing equal acceptability of *Lemna* incorporated diets to that of control diet.

i. Fish growth with *L. minor* incorporated supplementary diets

The final body weight (g) in different treatments increased from 8.53 to 13.90 in C, 8.25 to 16.70 in D1, 8.64 to 13.80 in D2, 8.38 to 14.00 in D3, 8.25 to 12.90 in D4 and 8.50 to 11.00 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (16.70), followed by D3 (14.00), C (13.90), D2 (13.80), D4 (12.90) and D5 (11.00) respectively and the differences were significant (D1>D3=C=D2≥D4≥D5). %NWG, SGR and PER was maximum in D1 (102.42, 0.39 and 1.93) and minimum in D5 (29.41, 0.14, 1.50). Likewise FCR was minimum in D1 (1.89) and maximum in D5 (2.56) showing maximum feed efficiency of diet D1. Overall results revealed that sundried *L. minor* can be incorporated in carp diet up to 40 % level without having any negative impact on fish growth; however, best results in terms of fish growth were recorded at 10 % incorporation level, which resulted in 20.14 % higher growth in terms of body weight.

ii. Fish growth with *L. gibba* incorporated supplementary diets

The final body weight (g) in different treatments increased from 8.53 to 13.90 in C, 8.25 to 14.50 in D6, 8.25 to 12.70 in D7, 8.25 to 10.70 in D8, 8.25 to 10.10 in D9 and 8.42 to 9.70 in D10. At the termination of the experiment, average final body weight (g) of fish was maximum in D6 (14.50), followed by C (13.90), D7 (12.70), D8 (10.70), D9 (10.10) and D10 (9.70) respectively and the differences were significant (D6=C=D7>D8=D9=D10). %NWG, SGR and PER was maximum in D6 (75.75, 0.31 and 1.69) and minimum in D10 (15.20, 0.08, 1.04). Like7wise FCR was minimum in D6 (2.14) and maximum in D10 (3.54) showing maximum feed efficiency of diet D6. Overall results revealed that sun dried *L. gibba* can be incorporated in carp diet up to 20 % level without having any negative impact on fish growth, however, best results in terms of fish growth were recorded at 10 % incorporation level, which resulted in 4.32 % higher growth in terms of body weight.

L. minor* Vs. *L. gibba

Of the two duckweed species tested, sun dried ***L. minor* was found to have more potential** for utilization as non-conventional feed resource in carp fingerling feed (up to 40 % incorporation level) with additional dual benefit in terms of feed cost reduction and fish growth enhancement at 10 % incorporation level. In contrast, sun dried *L. gibba* was found suitable for incorporation in carp diet up to 20 % level only, with additional dual benefit in terms of feed cost reduction and fish growth enhancement at 10 % level.

Experiment study 2: Efficacy of *L. minor* incorporated supplementary diets for carps in semi-intensive culture system

Experimental study was conducted to assess the efficacy of sun dried duckweed (*L. minor*) in the diet of carps viz. rohu, *Labeo rohita* and common carp, *Cyprinus carpio* under semi-intensive culture system for 180 days. Five duckweed based experimental diets were prepared by incorporating *Lemna* powder @ 10, 20, 30, 40 and 50 % (D2 to D6) respectively by replacing basal (control) diet (D1). Carp fingerlings were stocked in 20 m2 outdoor cemented tanks @ 1/m2 and were fed with different diets @ 5 % and 2% of their body weight daily for 60 and 120 days respectively. Observations were recorded in term of water quality parameters (temperature, pH, dissolved oxygen, total alkalinity, hardness, ortho-phosthate, ammonical nitrogen and nitrate nitrogen analyzed at fortnightly intervals), fish growth and flesh quality (total protein, total lipids, total carbohydrates, moisture and ash contents).

Water quality: Optimum water quality is required for optimum growth of fish under controlled conditions. In the experimental present study, the water temperature (29.07-29.39°C), pH (8.60-8.73), dissolved oxygen (6.97-7.49 mgl^{-1}), total alkalinity (167.08-184.62 mgl-1), total hardness (174.46-200.92 mgl^{-1}), orthophosphate (0.185-0.216 mgl^{-1}), ammonical nitrogen (0.012-0.122 mgl^{-1}) and nitrate nitrogen (0.134-0.159 mgl^{-1}) were well within the recommended range in all the treatments for supporting optimum growth in carps throughout the culture period and the differences among treatments were insignificant.

Fish Survival: At the termination of the experiment, 100 % survival of *L. rohita* and *C. Carpio* was recorded in all the treatments and control showing equal acceptability of *Lemna* incorporated diets to that of control diet.

Fish Growth

Labeo rohita

The final body weight (g) in different treatments increased from 1.12 to 35.70 in C, 1.09 to 36.40 in D1, 1.13 to 30.70 in D2, 1.11 to 29.00 in D3, 1.11 to 26.75 in D4 and 1.13 to 26.20 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (36.40), followed by C (35.70), D2 (30.70), D3 (29.00), D4 (26.75) and D5 (26.20) respectively and the differences were significant (D1=C>D2=D3≥D4=D5). %NWG, SGR ad PER was maximum in D1 (3248.67, 1.95 and 1.96) and minimum in D5 (2218.58, 1.74 and 1.81). Likewise FCR was minimum in D1 (1.87) and maximum in D5 (2.12) showing maximum feed efficiency of diet D1. Overall growth results showed 10 % incorporation level of *L. minor* as best among all other treatments and control.

Cyprinus carpio

The final body weight (g) in different treatments increased from 1.54 to 55.00 in C, 1.53 to 56.70 in D1, 1.48 to 38.90 in D2, 1.41 to 38.80 in D3, 1.51 to 40.60 in D4 and 1.51 to 41.30 in D5. At the termination of the experiment, average final body weight (g) of fish was maximum in D1 (56.70), followed by C (55.00), D5 (41.30), D4 (40.60), D2 (38.90) and D3 (38.80) respectively and the differences were significant (D1=C>D2=D3=D4=D5). %NWG, SGR ad

PER was maximum in D1 (3605.88, 2.01 and 2.09) and minimum in D2 (2218.58, 1.81 and 1.72). Likewise FCR was minimum in D1 (1.75) and maximum in D5 (2.17) showing maximum feed efficiency of diet D1. Overall growth results showed 10 % incorporation level of *L. minor* as best among all other treatments and control.

Significantly higher growth in terms of final body weight, NWG %, SGR and PER along with minimum FCR was recorded in both the fish species with *L. minor* supplemented diet (D1) up to 10 % incorporation level. Fish growth declined with further incorporation of *L. minor*.

Flesh Quality

L. rohita

The flesh protein content (%) was maximum (15.53) in D1 and minimum (14.07) in C, but the protein content in different treatments did not differ significantly. Maximum total lipid (%) was recorded in D2 and D3 (2.69) and minimum in C (2.10) and the lipid content among treatments did not differ significantly. The total carbohydrate content (%) was maximum (3.80) in D4 and minimum (2.10) in D1andthe difference among the treatments were not significant. Ash content (%) was maximum (1.78) in D2 and minimum (1.06) in D1and the difference among treatments were significant (D2=D3=D4≥C≥D5=D1). The flesh moisture content (%) was maximum (79.51) in C and minimum (77.38) in D4 but the moisture content in different treatments did not differ significantly.

C. carpio

The flesh protein content (%) was maximum (14.45) in D1and D2, and minimum (13.87) in C, but the protein content in different treatments did not differ significantly. Maximum total lipid (%) was recorded in D1 (3.43) and minimum in C (2.78) and the lipid content among treatments did not differ significantly. The total carbohydrate content (%) was maximum (2.57) in C and minimum (2.12) in D2 and the difference among the treatments were significant (C=D4=D1>D5=D3=D2). Ash content (%) was maximum (2.61) in D3 and minimum (1.29) in C and the difference among treatments were significant (D3=D4=D5≥D2≥C=D1). The flesh moisture content (%) was maximum (79.25) in C and minimum (77.50) in D4 but the moisture content in different treatments did not differ significantly.

Overall results of the experimental studies revealed that dried *Lemna* powder can be incorporated in carp diet @ 10 % by replacing conventional feed ingredients without compromising fish growth and flesh quality. Hence, *L. minor* can be incorporated in carp diet @ 10 % level by replacing basal diet ingredients.

Conclusions

S. No.	Results	Conclusion/Recommendations
1.	Suitable duckweed species w. r. t. growth response under culture conditions throughout the year, no. of harvestings, biomass productivity, winter tolerance and nutritive value	***L. minor***
2.	Best manure w. r. t. productivity	Poultry droppings
3.	Best dose of poultry droppings w. r. t. Productivity	600 kg/ha/wk
4.	Best manure w. r. t. nutritive value	Cow Dung : Poultry droppings (1:1)

5.	Best dose of Cow Dung : Poultry droppings (1:1) w. r. t. nutritive value	600 kg/ha/wk
6.	Best months w. r. t. productivity	March, May, July, August & September
8.	Best duckweed species for incorporation in carp feed	***L. minor***
9.	Sun dried *L. minor* incorporation level in carp fingerling diet	Up to 40 % level with additional dual benefit at 10 % incorporation level with 7 % reduction in feed cost and over 20 % enhancement in fish growth
10.	Sun dried *L. minor* incorporation level in carp fry diet	Up to 10 % incorporation level

- ***L. minor*** culture can be taken up under local climatic conditions of Punjab by using organic manures like **Poultry droppings** (@ 600 kg/ha/wk) and combination (1:1) of **cow dung and poultry droppings** (@ 600kg/ha/wk) during **pre-monsoon and monsoon months**.
- Although, the harvested biomass can be utilized as feed resource in carp feed i.e., **up to 40% in fingerling diet** (27.7% feed cost reduction) and up to **10% in fry diet** (7% feed cost reduction), but it is **recommended for incorporation in fingerling diet** for higher economic returns in terms of both feed cost reduction from 7 to 27.7% (up to 40% incorporation level) and 20% higher fish growth (at 10% incorporation level).