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Effect of partial replacement of fish meal with duck weed (*Lemna Minor*), and soybean meal on the growth performance of *Ctenopharyngodon Idella* (grass carp)

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Abstract
The acceptable nutritional value of *Lemna Minor* & soybean meal as an ingredients in diets for *Ctenopharyngodon Idella* (Val.) Fingerlings was experimented under aquarium culture system for 120 days. Six formulated experimental diets were prepared to contain 0%, 20% 40%, 60% 80% and 100% of gradient duck weed meal (diet DSM 0, DSM 20, DSM 40, DSM 60 DSM80 and DSM 100, respectively). Significant differences ($P<0.05$) in weight gain, specific growth rate, total feed intake, feed conversion ratio, and protein efficiency ratio were found among the feeding treatments, except for final survival rate. These results suggest that duckweed diet appears to be more adequate for a better growth of this fish.

Keywords: *Ctenopharyngodon idella*, *Lemna minor*, fish meal, fingerlings, growth

Introduction
In recent years a number of investigations were conducted on fish Polyculture in world using animal wastes to supplement pelleted fish feed, thereby reducing fish production costs, thereafter, investigations were carried out on the integration of fish and vegetable production, and a duck-fish vegetable integrated system [19]. The success of commercial aquaculture operations depends mainly on one key biological component that is the availability of suitable diets which provide required nutrients for optimum growth. As a result, use of supplementary feed has become inevitable for the carrying capacity of culture systems and can enhance fish production by many folds. One important aspect in common carp farming is to identify economically viable and easily available ingredients for formulating diets that are nutritive palatable and having maximal conversion ratio to give greater fish yield per application. Feed conversion ratio (FCR) values of various fish feeding ingredients for carps under controlled conditions have been estimated by many workers [1, 4].
The grass carp, *Ctenopharyngodon idella* is a fin fish and biological control agent for hydrilla (*Hydrilla verticillata* (L.f.) Royle) and other aquatic plants. Fingerlings are resembles with small adults. The grass carp is highly adaptable and can survive in a variety of conditions, the natural grass carp life cycle has not been observed to occur many times outside of the native range. The status of introduced grass carp populations is often difficult to determine because stocked individuals live such a long time and frequently there is little monitoring for successful recruitment. The grass carp is a grazer, feeding on vegetation mostly near the surface and in shallower waters. The new growth of submersed plants is preferred. Host preference is dependent on fish size, with small fish preferring musk grass (*Chara* spp.) and large fish preferring Hydrilla [6]. However, the grass carp is a generalist, and in the absence of the preferred host plant, will feed on most other types of aquatic vegetation. Grass carp even have been observed to feed on terrestrial plants that are hanging over the water. The five most-preferred species in order of preference are hydrilla, musk grass, pondweeds (*Potamogeton* spp.), southern naiad (*Najas guadalupensis* [Spreng] Magnus), and Brazillian elodea (*Egeria densa* Planch Anderson) [6]. Grass carp are not a good control method for filamentous algae, Eurasian milfoil (*Myriophyllum spicatum* L.), spatterdock (*Nuphar advena* Aiton), fragrant waterlily (*Nymphaea odorata* Aiton), sedge (*Cladium* spp.), cattail (*Typha* spp.), or other large aquatic plants [7].

Duckweed are small floating aquatic plants, widely available in India and consists of four genera viz. *Lemna, Spirodela, Wolffia and Wolffiella* among which about 40 species have been identified so far [5]. In India, it can easily be grown abundantly with minimum cost, made available in much cheaper than other alternative plant protein sources. Fresh duckweed is widely used as fish feed but only a few reports are available on the use of dry duckweed as fish feed.

Feedstuffs derived from soybean have been used predominantly for many years in diet formulations for the aquacultural production of numerous fish species. As a group, various freshwater fish species that exhibit omnivorous feeding behavior historically have been fed prepared diets containing relatively high levels of soybean meal (up to 60% by weight) [20]. This group of fish constitutes the largest sector of world aquaculture production by tonnage, and are a major user of soybean products [21]. In the present study, Feed was formulated by soyabean meal as plant protein sources. The other ingredients such as milk powder, corn flour, eggs, cod liver oil, vitamin mixture containing vitamin B Complex and E, agar powder, garlic paste, pepper powder, and cumin powder were also used. According to Nekoubin H *et al*. [19], determination of the required dietary protein levels is important to get highest growth and reduce the water deterioration problems related with supplementary feed intake of fish.

A length-weight relationship provides information on growth patterns and growth of fish. During their development, fish are known to pass through stages in their life history, which are defined by different length-weight relationships. It is applicable for basic needs in order to make fish stocks assessment and population.

The present investigation was designed to evaluate the suitability of duckweed as dietary fishmeal substitute for grass carp (*Ctenopharyngodon Idella*) fingerlings. Studies on growth performance of cultured fry of common carp in relation to feeding provide information for successful application in the management and exploitation of resources. The present trial was undertaken to quantitatively analyze the comparative efficacy of different formulated feeds of plant and animal origin in relation to growth of fry of *Ctenopharyngodon Idella*. The feeds selected for this investigation are fish meal Lemna minor meal of plant origin. Duckweed meal has been known for its high nutritive value with as much as 40% and above crude protein depending on the culture system.

Materials and Methods

Materials: Fresh Duckweed (*Lemna minor*) was harvested from local agricultural field. The freshly collected duckweed, selected quantity of duckweed was sun-dried. The dried duckweeds were gathered and grounded into fine particle and sieved through a mesh size of 2 mm and stored in a polyethene bag before been used for feed formulation. Other feed ingredients used for the formulation of the diets include Fish meal (g), Maize (g), Groundnut cake (g), Duckweed meal (g), Soybean (g), Bone meal (g), Methionine (g), Lysine (g), Vitamin C (g), Vitamin B complex (g), Salt (g). Soyabean meal was taken in powder form as main ingredient. The strands were cut into short pieces sun dried for three days to avoid fungal infection. The feed formulation was done using Pearson square method. The feed ingredients were grounded/drilled and weighed into a bowl with the use of a sensitive weighing balance. The maize grain and locally extracted groundnut cake were drilled separately into fine particles with the use of a milling machine.

Six diets were formulated for the *Ctenopharyngodon Idella* finger lings as shown in Table 1 with the cost of each diet. The milled feed ingredients were weighed and mixed in a bowl, then followed by a wet mixing using about 20 ml water per kilogram of feedstuff to form tough-dough. The resulted dough was extracted through an electrical powered pelleting machine into suitable pellet size. The pellets were collected and spread out to dry under the sunlight or under a fan at a room temperature (23 °C) for 24 hours. The pellets were then broken into bits, packaged and stored in a cellophane bag until it was to be used.

Table 1: Ingredients and proximate composition on a dry weight basis of six experimental diets

Ingredients (%)	DSM0	DSM20	DSM40	DSM60	DSM 80	DSM 100
Fish meal(g)	18	18	18	18	18	18
Maize (g)	26	26	26	26	26	26
Groundnut cake (g)	20	18	18	18	18	18
Duckweed meal (g)	0	16	16	16	16	16
Soybean (g)	28	14	14	14	14	14
Bone meal (g)	1	1	1	1	1	1
Methionine (g)	1.4	1.4	1.4	1.4	1.4	1.4
Lysine (g)	1.4	1.4	1.4	1.4	1.4	1.4
Vitamin C (g)	1.4	1.4	1.4	1.4	1.4	1.4
Vitamin B complex (g)	1.4	1.4	1.4	1.4	1.4	1.4
Salt (g)	1.4	1.4	1.4	1.4	1.4	1.4
Total	100	100	100	100	100	100
Cost (Rs)	2.3	2.09	2.09	2.09	2.09	2.09

DSM0=Fishmeal 100% and Duckweed meal0%; DSM20=Fishmeal 80 % and Duckweed meal 20%
DSM40=Fishmeal 60% and Duckweed meal 40%; DSM60=Fishmeal 40 % and Duckweed meal 60%
DSM80=Fishmeal20% and Duckweed meal 80%; DSM100=Fishmeal 0% and Duckweed meal 100%

Water Quality: Water quality parameters (temperature, dissolved oxygen, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for *Ctenopharyngodon Idella*. Water temperature and dissolved oxygen were measured every other day using an Elico-India model water analyser. Ammonia and nitrite were measured at weekly intervals. Alkalinity was monitored twice weekly using the titration methods of Golterman *et al*. [9] pH was monitored twice weekly using an electronic pH meter (Elico-Made). During the feeding trial, the water quality parameter averaged (± SD): water temperature 26.9 ± 0.5˚C dissolved oxygen 5.2 ± 0.2 mgl^{-1};

pH 7.2 ± 0.4; ammonia 0.01 ± 0.03 mgl^{-1}; nitrite 0.1 ± 0.05 mgl^{-1}; nitrate 1.5 ±.2 mgl^{-1}; alkalinity 127 ± 46 mgl^{-1}.

Experimental

Studies were conducted on *Ctenopharyngodon idella* (Figure 1), Fish (200 samples) weighing 16.27±0.001 gm with body length 12.22±0.20 cm and kept in aquarium for seven days of acclimatization. Then they were randomly selected and experiment was conducted in five glass aquaria has dimension (45×46.5×45 cm^3) with water volume 60 liter under continuous aeration and experimental set up were under natural light and dark cycle. For each feed i.e. conventional and different combination of feed three replicates were used and in each replicate 25 fishes were stocked. They were fed at the rate of 2% of live net weight once in a day. A water change was implemented by replacing one half of the water in each aquarium with dechlorinated water every day. These pooled samples were used for further studies [8].

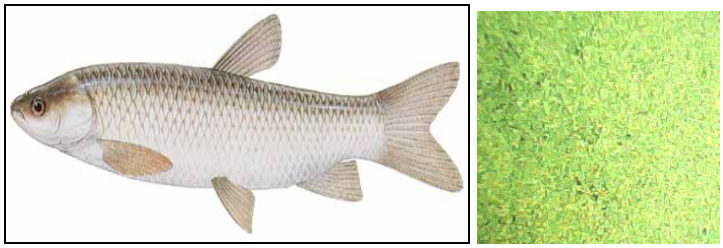

Fig 1: *Ctenopharyngodon idella* and Duck weed meal (left)

Feeding rate and frequency: Fingerlings were fed with 5% of their body weight for 120Days. The pellets were reduced to suitable size with the use of a laboratory ceramics mortar and pestle and fish were fed twice daily (morning and evening).

Twenty five *Ctenopharyngodon Idella* fingerlings were evenly distributed in each of the aquarium. At the end of the experiment, the growth performance was assessed by determination of feed consumption (FC), weight gain (WG), absolute growth rate (AGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate as described by Hernandez *et al*. [11] as follows:

1. Mean Weight Gain (MWG)=Mean final weight - Mean initial weight
2. Specific growth rate (SGR) = 100 ($\ln W_2 - \ln W_1$) / T, where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the experimental period
3. % Survival rate=(total number of fish – mortality) / total number of fish×100
4. Feed efficiency ratio (FER) = weight / feed intake
5. Feed conversion ratio (FCR)= dry feed intake / wet weight gain
6. Protein efficiency ratio (PER) = wet weight gain / protein fed

Where Protein fed= % protein in diet × total diet consumed / 100

Condition factor (k)

This expresses the health status of fish as a result of the experimental treatment and was computed at the beginning and end of the experiment using the Fulton's Condition Factor Formula as expressed by Bagenal and Tesch [12] as:

$k=100\ W/L^{-3}$

Where W=weight of fish

L = length of fish

Statistical analysis: Statistical analysis of data was performed by analysis of variance (ANOVA) using Microsoft Software statistic followed by Duncan's multiple range test [10]

Proximate analysis

The proximate analysis of all the experimental diets for crude protein, crude fibre, ash and moisture content was carried out following the method of Association of Official Analytical Chemical [11].

Table 2: Chemical analysis of Experimental diets

Composition	DSM0	DSM20	DSM40	DSM60	DSM 80	DSM 100
Dry matter	97.45	97.13	97.06	97.00	97.91	97.79
Moisture Crude	2.55	2.87	2.94	3.00	2.89	3.02
Crude Protein	29.28[b]	36.25	28.62[b]	30.04	36.54	30.28
Total lipid	11.85	12.29	11.93	12.70	12.71	12.66
Crude Fibre	16.15	13.76	15.26	16.96	13.87	15.08
Ash	11.1	10.78	11.41	11.5	11.54	11.37
Gross energy *(KJ g^{-1})	19.75	19.92	19.63	19.84	19.91	19.95

* Calculated using combustion values for protein, lipid, and carbohydrate of 23.6, 39.5, and 17.2 kJ g−1, respectively. Carbohydrate was calculated by the difference: 100 − (protein + lipid + ash + moisture). Tables 1 and 2 show the composition and chemical analysis of the experimental diets.

Results and Discussion

There were no significant differences in water quality parameter among the treatments during experimental period. Water quality parameters were within the acceptable range for *Ctenopharyngodon Idella* growth rate.

Growth Parameters and Nutrient Utilization

Growth performance and feed utilization of the fish are given in Table 3 and Figure 2. The highest growth performance in terms of Final body weight (33.01± 0.07g fish-1) in DSB 60, Mean Weight Gain (18.73 g fish-1) in DSM 20 and Specific growth rate for weight (1.43% day^{-1} $fish^{-1}$) was observed in DSM20 type meal on the fish fed. Survival (%) of fish did not differ significantly (P>0.05) among treatments. Final weight, weight gain (%), specific growth rate (SGR), and Protein efficiency ratio of fish fed DSM 20 diet was significantly

higher than those fed the other diets. On the other hand, the growth parameters of fish fed DSM 60, and DSM 80 were not significantly different from those of fish fed DSM 100, DSM 40. The poorest growth performance was found in fish fed DSM 100. However, no difference was detected in feed efficiency ratio (FER) and protein efficiency ratio (PER) between DSM 60, DSM 80 and other dietary groups.

Table 3: Growth parameters and nutrient utilization in duckweed/soya bean fed test diets for 120 days

Parameters	DSM0	DSM20	DSM40	DSM60	DSM 80	DSM 100
Initial body weight (g/snail)	16.27 ± 0.01	16.27 ± 0.01	16.27 ± 0.01	16.27 ± 0.01	16.27 ± 0.01	16.27 ± 0.01
Final body weight (g/fish) (FBW)	31.24 ± 0.25	32.0± 0.18	32.05± 0.23	33.01± 0.07	32.54± 0.07	32.88± 0.08
Mean Weight Gain (MWG)	14.97	18.73	15.78	16.74	16.27	16.61
Initial length (cm)	12.22 ± 0.20	12.12 ± 0.21	12.02 ± 0.20	12.20 ± 0.21	12.20 ± 0.21	12.21 ± 0.22
Final length (cm)[(a)]	17.7± 0.20	19.84± 0.20	19.14± 0.20	19.8± 0.20	19.84± 0.20	17.84± 0.20
Body length increased (cm)	5.48	7.62	6.92	7.58	7.62	5.62
Specific growth rate for weight (% $BWday^{-1}$)	1.03 ± 0.0[b]	1.43 ± 0.02[c]	1.38 ± 0.01[a]	1.40 ± 0.05[c]	1.41 ± 0.03[d]	1.08± 0.03[d]
Feed Conversion Ratio (%)	42.15 ± 0.11[b]	81.24 ± 0.02[a]	78.24 ± 0.22[c]	79.22 ± 0.01[c]	80.01 ± 0.01[b]	44.15 ± 0.01[b]
Feed Conversion efficiency (%)	0.3 ± 0.01[b]	0.8 ± 0.01[b]	0.6 ± 0.0[a]	0.7 ± 0.0[a]	0.6± 0.01[b]	0.35± 0.01[b]
Condition Factor	0.65 ± 0.01[b]	0.7 ± 0.03[c]	0.9 ± 0.06[a]	0.69 ± 0.01[b]	0.82 ± 0.02[d]	0.82 ± 0.02[d]
Daily growth ratio (g)	0.11 ± 0.0[b]	0.19 ± 0.01[c]	0.17 ± 0.01[a]	0.16 ± 0.01[c]	0.18 ± 0.0[d]	0.12 ± 0.0[d]
Survival rate (%)	100	100	100	100	100	100

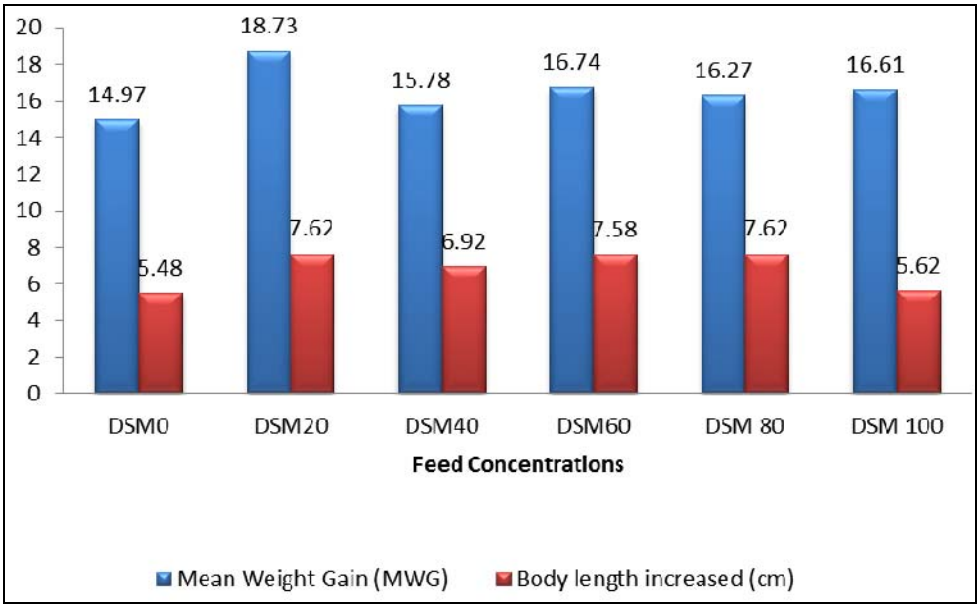

Fig 2: Variations in weight gain and Body length increased as per variations in feed concentration in 120days

The result of body composition showed that using of formulated diet had not significantly affect quality of whole body values like protein, moisture and ash. However, the highest protein content was obtained in *DCM80*. But fed formulated diet showed significant increase in lipid value. The variation in the nutrients especially in crude protein of the duckweed with different concentrations is emphasizing the effect of treatments on protein molecules. Protein requirement is given high priority in any nutritional study because it is the single nutrient that is required in the largest quantity for growth and development and also the most expensive ingredient in diet formulation [13]. The duck weed composition of the experimental fish as presented in Table 2 shows that there were significant variations in the moisture, protein fat and Ash content levels of the fish fed the different experimental diets. However while moisture and fiber was observed higher in DSM 60, protein and fat were on the other hand higher in DSM 80 ($P<0.05$).

Jobling [14] believed the evacuation of high energy density artificial diet was slower than prey in natural environment, which was also confirmed by the present study which demonstrated that the intestinal evacuation time of formulated diet (12h) is 3 times longer than natural food of grass carp.

Therefore, making a proper diet is essential to improve the efficiency of digestion and absorption, which this in turn causes getting enough energy and protein. Thus, in this research we have used duck weed and soya bean diets with different protein percentage, and it was clearly observed that treatment (case) fed in a diet with high protein (36.5%) had better growth performance and less food conversion ratio (FCR) than the other case under a diet with low protein (25%). Also the highest FCR was obtained in formulate diet with low protein similar result ($P<0.05$) reported by Ghazala *et al*. [15] Hossain *et al*. [16] and Gao *et al*. [17].

Duck weed composition of the fingerings fed all the various diets had more protein retained in the body at the end of the experiment. This showed that the protein to energy ratio used in the feed was at the right proportion as a result, there was no sparing of protein for energy [18]. A confirmation of the good quality can be seen in the reduction in fat content of the fingerings fed the various diets when compared to result of the initial fat content of the fingerlings. This result to the non-fatty fleshed fish produced. The present study has therefore shown that *Duckweed* meal can replace fishmeal up to 50%

without affecting growth and nutrient utilization significantly, however beyond this growth and stored protein reduces as the level of inclusion increases.

Conclusion

The study clearly showed that fish fed diet with 20% duck weed perform excellently well compared to other compositions. Further aquatic weed based feeds are cheaper as well as simpler compared to other conventional feeds of aquatic weeds in Grass carp (*Ctenopharyngodon idella*) diets would also prove economically viable.

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