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Duckweed (*Lemna minor*) as a plant protein source in the diet of common carp (*Cyprinus carpio*) fingerlings

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Abstract
The present study was carried out to use dried duckweed, *Lemna minor*, as a dietary protein source for (*Cyprinus carpio*) common carp fingerlings. Four experimental diets were prepared with similar E:P ratios were fed to common carp fingerlings with an average initial weight of 16.0 g for 60 days. A diet containing 15%, 30% and 45% duckweed respectively was substituted for the commercial 32% protein control-group diet, fed in normal rations to common carp. The results revealed that there was no significant difference between the growth performance of fish that were fed diets containing up to 45% duckweed and fish that were fed the control diet ($P>0.05$), except for the group of fish on the 15% duckweed diet. Also, no significant difference was observed among treatments with respect to feed utilization ($P>0.05$). While carcass lipid content increased, protein content of the fish fed a diet of 15% duckweed increased compared to other groups ($P<0.05$). The results showed that a diet consisting of up to 15% content could be used as a partitial replacement for fish meal in the diet of common carp fingerlings.

Keywords: Fish meal, duckweed, common carp, plant protein

Introduction
Fish culture is gaining importance day by day in Jammu and Kashmir State. Good number of fish farms has come up in the state for the last few years. In order to provide cost effect feed for private fish farmers, use of locally available feed ingredients needs to be exploited. There is a lot of scope for non-conventional fish feed ingredients also like duckweed, silkworm pupae as protein sources. In order to formulate feed for fish, one must have knowledge on nutritional requirements of fish for good growth and also is able to identify different sources of feed ingredients that ultimately make up the diet. These ingredients have first to be evaluated to determine their suitability and safety for use in compounded feed. Precise information on the chemical composition of individual feed ingredients is indispensable, not only for formulation of efficient diets that meet desired nutrient specifications but also for setting restrictions for their safe usage when anti-nutritional factors are known to be present.

Duckweed, as a natural protein source, has a better array of essential amino acids than most other vegetable proteins and more closely resembles animal protein (Hillman and Culley, 1978) [10]. Newly harvested duckweed plants contain up to 43% protein by dry weight and can be used without further processing as a complete feed for fish. Compared with most other plants, duckweed leaves contain little fiber (5% in dry matter for cultivated plants) and little to no indigestible material even for monogastric animals (Chaturvedi *et al.*, 2003) [3]. Duckweeds have been fed to animals and fish to complement diets, largely to provide a protein of high biological value. Fish production can be stimulated by feeding duckweed to the extent that yields can be increased from a few hundred kilograms per hectare/year to 10 tonnes/ha/year (Leng *et al.*, 1994) [12]. A major limitation to fish farming is that meals high in protein with high biological value are expensive and often locally unavailable. Fresh duckweed is highly suited to intensive fish farming systems with relatively rapid water exchange for waste removal (Gaigher *et al.* 1984) [8] and duckweed is converted efficiently to live weight by certain fish including carp and tilapia (Hassan and Edwards 1992) [9]. Keeping in view the nutritional value of duckweed, present study was conducted to ascertain its potential as a protein supplement in the diet of common carp fingerlings.

Materials and Methods

Diet formation and preparation

Freshly harvested duckweed from the Dal Lake, Srinagar, and Kashmir was collected and sun dried for four days. After initial grinding, it was again dried in hot air oven for 12 hrs at 45^0 C and thereafter grounded into fine powder using the simple grinder. All the other basal feed ingredients were thoroughly grinded and sieved through a 595um sieve to ensure homogeneous size profile before analysed for proximate composition.

Four dry diets were prepared in which fish meal was replaced with duckweed meal at 0%, 15%, 30% and 45% levels using the method of Akegbejo Samson (1999) [2] at 32% crude protein level. The diets were fortified with vitamin premix. They were thoroughly mixed in a bowl and pellets were made through hand pelletizer.

Table 1: Percentage Composition of experimental feed

	Percent inclusion of duckweed			
Ingredient	**Control (0%)**	**Treatment 1 (15%)**	**Treatment 2 (30%)**	**Treatment 3 (45%)**
Duckweed	0	3.9	7.8	11.7
Fish meal	26.5	22.6	18.7	14.8
Mustard oil cake	30.0	30.0	30.0	30.0
Rice bran	21.0	21.0	21.0	21.0
Wheat bran	6.5	6.5	6.5	6.5
Vegetabel Oil	15.0	15.0	15.0	15.0
Vitamin & Mineral Mix.	1.0	1.0	1.0	1.0
Total	100	100	100	100

Experimental Site

The experimental study was conducted at the wet laboratory of fish farm of Faculty of Fisheries, Rangil, and SKUAST-Kashmir.

Experimental system and feeding trial

The Feeding trial was conducted in plastic tubs (30 x 30 x 60cm^3). The plastic tubs were properly washed and rinsed with clean water. They were filled with borehole water and aerated using air pumps to ensure proper oxygenation and continual aeration. The experimental fish *Cyprinus carpio* fingerlings were collected from the Shuhama fish farm of Faculty of Fisheries, SKUAST-Kashmir. They were acclimatized for five days before the commencement of the experiment. The fishes were acclimatized to enable them to bear new conditions and empty their stomach content.

Experimental Procedure

The experimental fish were randomly distributed at a stocking density of 10 fingerlings per plastic tub in triplicates. They were fed at 5% body weight twice daily morning and evening at split dose. Sampling was done weekly using a sensitive electronic balance (OHAS – LS – 500g Model) to determine the average weight of the fish and adjust the feed accordingly. The study was conducted for 60 days. All analyses for proximate composition including the carcass composition before and after the experiment were determined according to the methods of AOAC (2000). Water temperature was monitored daily with a standardized mercury thermometer while dissolved oxygen and PH were determined using Digital DO meter and Jenway Automatic PH meter (Jenway 3015) respectively.

Table 2: Proximate composition of experimental feeds

Chemical composition	Control (0%)	Treatment 1 (15%)	Treatment 2 (30%)	Treatment 3 (45%)
Dry matter	87.0	87.7	88.2	88.5
Crude protein	21.82	21.42	20.67	20.00
Crude fibre	6.82	7.03	7.07	7.21
Ash	14.91	15.79	16.21	16.65
Lipid	12.64	11.43	10.82	10.23
NFE	31.82	33.31	34.22	34.89
Gross energy	425.95	412.57	406.20	409.53

Measurement of growth parameters

Food conversion ratio (FCR), specific growth rate (SGR) and percentage survival rate were determined as follows (After Fagbenro *et al*., 1992) [7].

$$FCR = \frac{\text{Dry Weight Feed Supplied}}{\text{Total Weight gain by fish (g)}}$$

$$SGR\ (\%) = \frac{100\ (\text{Loge Wt.} - \text{Loge WO})}{\text{T (days)}}$$

Initial Body Weight =
Final Body Weight =

$$\text{Wt gain} = \frac{\text{Wt} - \text{Wo}}{\text{T (days)}}$$

Where Wt = final body weight of fish in grams at the end of the experiment.
WO = Initial body weight of fish in grams at the beginning of experiments.
Loge = Natural Logarithm of both final and initial body weight of fish in grams.
T = Duration (time) of the feeding trial in days.
No = Number of fingerlings alive at the end of experiment.
S = % survival

Protein efficiency ratio (PER)

This index use growth as a measure of nutritive value of dietary protein. At was determined using Wilson (1989) as

$$PER = \frac{\text{Mean weight gain (g)}}{\text{Mean protein intake (mg)}}$$

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to compare the result. Statistical tables were used to evaluate the difference between means for individual diets at 5% (0.05) significance level.

Results and Discussion

Fish diet development involves not only the application of knowledge on nutritional requirements for growth of the specified species but also identification and development of ready sources of feed ingredients that ultimately make up the diet. In the present study three-inclusion level of duckweed in the experimental feed supported the growth for *Cyprinus carpio.* However, growth performance and feed utilization was favoured by low inclusion level of duckweed in the experimental feed. These results are very much similar to the reports of several authors who have demonstrated the use of several species of duckweed as a partial replacement for fishmeal in the diet of fish and other animals. Effiong *et al*., (2009) [5] reported that inclusion of duckweed at 10% in the diet of *Heterobranchus longifilis* fingerlings gives better results as compared to diets containing duckweed at 20% and 30%. Fasakin *et al*., (2001) reported the use of duckweed *Spirodellla polyrrhiza* in the diet of the Nile Tilapia *(Oreochromis niloticus).* They stated that fish fed duckweed based diet had higher growth rates than fish fed diet containing water fern meals.

The authors indicated the possibility of partial replacement of fishmeal with duckweed in the diet of Nile Tilapia. Shireman *et al*., (1978) reported that grass carp performance on a duckweed diet was superior to fish maintained on catfish chow. Noor *et al*., (2000) reported that silver barb *(Barbodes gonionotus* Bleeker) fed with diet containing duckweed at 20% showed better results compared at higher inclusion levels. The present study revealed that duckweed inclusion level at lower level (15%) gives better results compared with higher inclusion levels (30% and 45%) which is in agreement with earlier workers. Robinette *et al*., (1980) fed channel catfish on prepared diet consisting of 20% dry duckweed; the weight gain, food conversion and energy use were equal to central diets (a standard catfish feed). Ahamad *et al*., (2003) reported also the replacement of sesame oil cake by duckweed in broiler diet. As per their results partial replacement of the costly oil seed by cheaper unconventional duckweed in broiler diet resulted in increase profitability.

Present findings are not in agreement with the work of Okoye and Mbagwu (1985) who observed higher FCR i.e. poor growth performance of *Sarotherodon galilaeus* fingerling fed diet containing 33% crude protein with 10% duckweed. Similar results were observed by Devaraj *et al.* (1981) to incorporate 40% *Lemna* powder with rice bran, oilcake and ragi flour in common carp diet resulted similarly poor growth and food utilization. The significantly lower growth of *Cyprinus carpio* observed with diet containing 30% and 45% duckweed may be due to the decreased level of fish meal and higher level of duckweed inclusion. The growth responses for fish fed diets containing increasing level of duckweed may presumably be due to the presence of anti-nutritional factors, low essential amino acids (EAAs) and polyunsaturated fatty acids, although no reports are available on the toxic substances present in duckweed. Although the EAAs content of the experimental diets were not analysed, another possible cause of retard growth may be that diets containing higher levels of duckweed were deficient in some of the EAAs. Duckweed is reported to contain very low amount of EAA (Hossain 1996) [11].

The protein efficiency ratio in the diets at 15% to 30% inclusive levels of duckweed in the experimental diet showed no significant difference compared with the control diet. Bairagi *et al*., (2002) reported that 30% fermented lemna leaf meal incorporated in the diet of *Labeo rohita* gave the best performance in terms of growth response, food conversion ratio and protein efficiency. From this result, 15% duckweed diet had the best specific growth rate and food conversation ratio compared to higher inclusion levels of duckweed.

Fasakin *et al*., (1999) [6] reported that there was no significant difference in growth performance and nutrient utilization of fish fed on diets containing up to 20% duckweed inclusion and the control. They further stated that increase in dietary duckweed inclusion resulted in progressively reduced growth performance and nutrients utilization of fish. This report is very much similar to the findings of the present study. Inclusion of duckweed meal in the diet of other animals to replace fishmeal or soybean has also been reported by Samnang (1999) [15]. The present results are in complete agreement with Patra (2015) who reported that 15% duck weed perform excellently well in *Labeo rohita* fry compared to treatments were higher inclusion rates have been given.

Table 3: Growth performance and feed utilization of *Cyprinus carpio* fingerlings fed duckweed based diet for 60 days.

	Duckweed substitution rate			
Parameter	**Control (0%)**	**Treatment 1 (15%)**	**Treatment 2 (30%)**	**Treatment 3 (45%)**
IBW(g)	16.27 ±0.01	16.20 ± 0.01	16.22 ± 0.01	16.25 ± 0.01 16
FBW(g)	22.24 ±0.25	22.05± 0.23	22.0± 0.18	23.01± 0.07
BWG (g)	5.97	5.85	5.78	5.78
SGR (%day-1)	1.43 ± 0.02^{c}	1.40 ± 0.05^{c}	1.38 ± 0.01^{a}	1.03 ± 0.0^{b}
FCR	2.41±0.11^{a}	2.45±0.14a	2.95±0.26a	2.45±0.19^{a}
PER	1.67±0.14^{a}	1.55±0.08^{a}	1.49±0.07^{a}	1.35±0.13^{a}
SR (%)	84.4	76.6	77.7	74.4

Data were presented as mean±SE (n=3).

Figures in the same row with same superscripts are not significantly different (P>0.05)

Conclusion

The present study revealed that duckweed (*Lemna minor*), which is almost costless, could be used to partially replace the very expensive fishmeal in the diet of *Cyprinus carpio* fingerlings at regulated inclusion levels. This will no doubt reduce cost of production but at the same time encourage fish farmers to take up fish culture on a large scale.

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