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Effects of duckweed (*Lemna minor*) as dietary fishmeal substitute for silver barb (*Barbodes gonionotus* Bleeker)

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Abstract
A 60-day long growth trial was conducted to evaluate the suitability of duckweed *Lemna minor* as dietary fish meal substitute for silver barb (*Barbodes gonionotus* Bleeker). Five iso-nitrogenous diets were formulated to contain 35% protein and each treatment had three replicates with 15 fish in each aquarium with a mean initial weight of 1.5 ± 0.2 g. Duckweed was used in the experiment to replace 10, 20, 30 and 35% of the dietary fish meal in diet 2, 3, 4 and 5 respectively. Fish meal was used as the sole source of protein in control diet (Diet 1). Fish were fed three times daily at satiation level. In terms of growth, food conversion and protein utilization, the control diet and diet containing 17.07% duckweed showed the best ($P<0.05$) performance followed by diets containing 34.14%, 51.21% and 59.24% duckweed. Fish fed diets containing higher levels of duckweed had higher carcass moisture and lower lipid content compared to the control diet. Histopathological examination revealed abnormalities in the liver of fish fed diets containing higher inclusion of duckweed. It was noted that 10% of the dietary fish meal protein could be replaced by duckweed (*L. minor*) in the diet of silver barb (*B. gonionotus*).

Key words: *B. gonionotus*, Duckweed, Fish meal

Introduction

Duckweed are small floating aquatic plants, widely available in Bangladesh and consists of four genera *viz. Lemna, Spirodela, Wolffia* and *Wolffiella* among which about 40 species have been identified so far (Journey *et al.* 1991). In Bangladesh, 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.

Silver barb, *Barbodes gonionotus* is one of the best suited species for bringing the unutilized or underutilized water bodies under intensive culture. This fish was also found to feed well on supplemental feed (Hussain *et al.* 1987). The fish can grow fast at high stocking densities (Sipitekhiat and Leenannod 1984).

The present investigation was designed to evaluate the suitability of duckweed as dietary fishmeal substitute for silver barb (*B. gonionotus*) fingerlings.

Materials and methods

A static indoor rearing system was used for the growth trial. Rectangular glass aquaria of 55 L capacity containing about 50 L of water was used as experimental tanks. Tap water was supplied in the aquaria during the experimental period and aeration was provided to maintain the adequate oxygen level. Induced bred fingerlings of silver barb (*B. gonionotus*) were obtained from a stocking pond adjacent to the Faculty of Fisheries at the Bangladesh Agricultural University (BAU) Campus, Mymensingh. Prior to the start of the experiments, the fingerlings were acclimated to the laboratory condition for seven days.

Five iso-nitrogenous diets were formulated to contain 35% protein to evaluate duckweed (*L. minor*) as dietary fish meal substitute for silver barb (*B. gonionotus*). The fish meal was prepared in the laboratory by grinding small dry fishes of mixed origin and seived to pass through 0.5 mm mesh. Duckweed were collected locally from a fish pond in a village adjacent to BAU campus. After collection, duckweed were dried in the sun and ground into powder to pass through a 0.5 mm seive. Before formulating the test diets, all the ingredients were subjected to proximate analysis and the results are presented in Table 1. Then the various ingredients were mixed together in required quantities according to formulations as shown in Table 2.

Table 1. Proximate composition of protein sources (% dry matter basis)

Protein sources	Dry matter	Protein	Lipid	Ash	C. fibre	NFE*
Fish meal	96.55	65.44	10.00	20.39	1.19	2.48
Duck weed	93.48	20.50	7.02	14.41	13.47	44.60

** NFE (Nitrogen free extract) calculated as 100 - % (moisture + protein +lipid +ash +crude fibre)*

Table 2. Formulation of experimental diets

	Diet No.				
Ingredients (%)	1(Control)	2	3	4	5
Fish meal	53.48	48.13	42.78	37.43	34.76
Duck weed	-	17.07	34.14	51.21	59.24
Cod liver oil	-	-	1.00	1.50	1.50
Soybean oil	5.00	4.00	3.00	1.50	1.00
Starch	28.00	23.00	13.58	2.86	1.00
Vitamin premix[1]	1.00	1.00	1.00	1.00	1.00
Mineral premix[1]	2.00	2.00	2.00	2.00	1.00
CMC[2]	2.00	2.00	2.00	2.00	1.00
α-Cellulose	8.02	2.30	-	-	-
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

1 According to Hossain and Jouncey (1989) [2]Carboxymethyl cellulose of high viscosity.

Adequate amount of water was added to moisten the mixture and the mixture was extruded through 1 mm diameter die of a pelleting machine (Hobart Mixture machine A 200). All the diets were subjected to proximate analysis and the results are presented in Table 3.

Table 3. Proximate composition of the experimental diets (% dry matter basis)

Parameters	Diet No.				
	1	2	3	4	5
Dry matter	91.86	91.93	92.68	93.09	92.05
Crude protein	33.88	33.85	34.67	34.28	34.39
Lipid	10.34	9.85	11.65	10.33	10.31
Ash	13.01	14.61	15.44	16.12	17.98
Crude fibre	7.05	6.48	6.98	8.08	8.03
NFE[1]	35.72	35.21	31.26	31.19	29.29
Gross energy (kcal/g)[2]	4.26	4.20	4.25	4.10	4.03
P/E ratio[3]	79.53	80.59	81.57	83.60	85.33

[1] *Nitrogen free extract =100-% (Crude protein + lipid + ash + crude fibre).*
[2] *Gross energy calculated after Jauncey and Ross (1982).*
[3] *Protein to energy ratio in mg protein /Kcal of total energy.*

The uniform sized fingerlings of silver barb were randomly distributed at the rate of 15 fish in each replicate with initial mean weight of 15±0.02 g. The fish were fed at satiation level with the formulated diets three times daily at 4 hourly intervals between 09.00 and 17.00 hours. In order to maintain good water quality, about half of the water in each tank was changed every day throughout the experimental period. Faeces were collected during the last two weeks of the experimental period for the study of protein digestibility of diet. Water quality parameters such as temperature, dissolved oxygen and pH were monitored weekly and the ranges were: temperature 27-30^{0}C, dissolved oxygen 6.2 - 8.3 mg/l and pH 6.8 - 7.8.

Feed ingredients, experimental diets and fish sample were analysed for their proximate composition (Horwitz 1980). The chromic oxide content of the experimental diet and faeces was determined after Furukawa and Tsukahara (1966). The histological study of different organs were done according to the procedure and methods described by Luna (1968). Specific growth rate (SGR), weight gain (%), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU) and apparent net protein digestibility (APD%) were calculated after Castell and Tiews (1980). Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (Duncan 1955) to test the significance of variation among the treatment means.

Results

In term of growth, food conversion and protein utilization, control diet and diet 2 (containing 17.07% duckweed) showed significantly ($P>0.05$) best growth performances among the experimental diets. Fish fed diet containing 59.24% duckweed produced significantly ($P>0.05$) the lowest growth performance. The growth performances of silver barb fingerlings during the experimental period are presented in Table 4.

Table 4. Growth and feed utilizition of *B. gonionotus* fed experimental diets

Parameters	Diets					
	1	2	3	4	5	± SE[2]
Initial wt. (g)	1.48[a]	1.50[a]	1.51[a]	1.52[a]	1.49[a]	0.02
Final wt.(g)	5.14[a]	5.06[a]	4.52[b]	3.65[c]	3.35[d]	0.038
Weight gain (g)	3.66[a]	3.56[a]	3.01[b]	2.13[c]	1.86[d]	0.034
% Weight gain	247[a]	237[b]	199[c]	140[d]	125[c]	2.46
SGR (% day)	2.07[a]	2.02[a]	1.83[b]	1.46[c]	1.35[d]	0.016
FCR	1.86[a]	1.92[a]	2.32[b]	3.21[c]	3.66[d]	0.045
PER	1.59[a]	1.54[a]	1.28[b]	0.91[c]	0.79[d]	0.033
ANPU (%)	25.53[a]	25.05[a]	19.42[b]	13.76[c]	11.69[d]	0.164
APD (%)	90.42[a]	88.60[b]	85.26[c]	81.24[d]	80.10[d]	0.43

[1] *Figures in the same row with same superscripts are not significantly different ($P>0.05$).*
[2] *Standard error of treatments calculated from the residual mean square in the analysis of variance.*

The highest SGR value was obtained from the control diet and diet 2 containing 17.07% duckweed (Table 4). The FCR values ranged between 1.86 to 3.66 with diet 1 and 2 producing significantly ($P<0.05$) the lowest FCR. The PER values ranged between 0.79 to 1.59. Diet 1 and 2 produced higher PER values of 1.59 and 1.54, respectively. There was no significant difference ($P>0.05$) between the ANPU values of control diet and diet containing 17.07% duckweed. The ANPU values of different diets ranged between 11.69 to 25.53%. Control diet produced significantly the highest APD value and diet 5 containing 59.24% duckweed produced the lowest APD value. In general, the APD value decreased with the decreasing level of fish meal in the diets.

Histological examination of control diet showed no evidence of any degenerative changes in any organs. Plate 1a shows the sections of liver of fish fed control diet. The most noticeable microscopic changes were recorded in the liver of the fish fed diets with higher levels of duckweed (59.24 %). The striking lesions of liver was the appearance of fat changes which was characterized by the presence of empty spaces in the hepatocytes (Plate 1b). There were also focal accumulation of leukocytes and haemmorhagic lesions in the midzonal area of hepatic lobules (Plate 2).

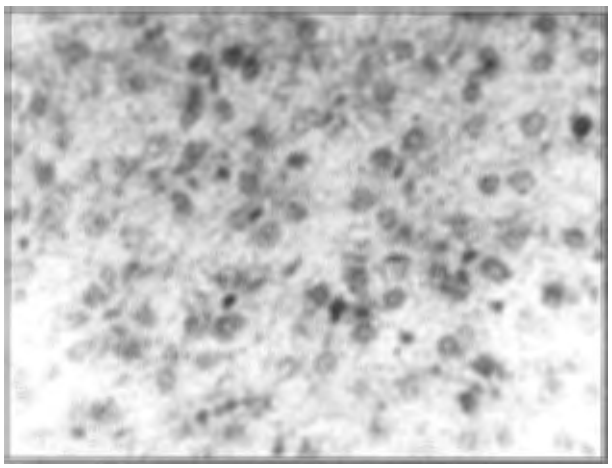

Plate 1a. Section of fish liver from control diet (400 times) showing no evidence of fatty changes.

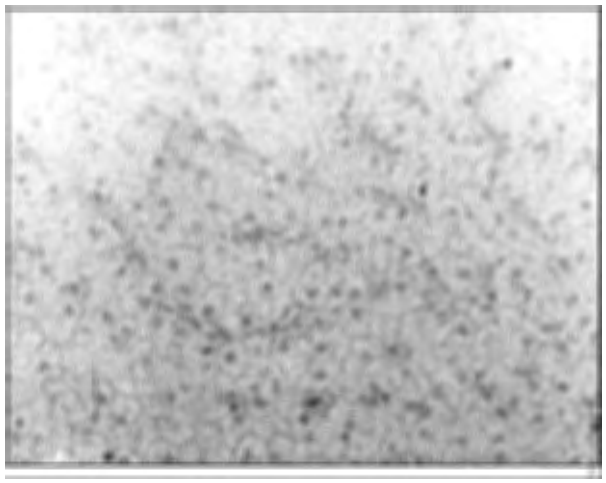

Plate 1b. Showing fatty changes in the cytoplasm of hepatic cells (400 times) in liver of fish fed diet containing 59.24% duckweed.

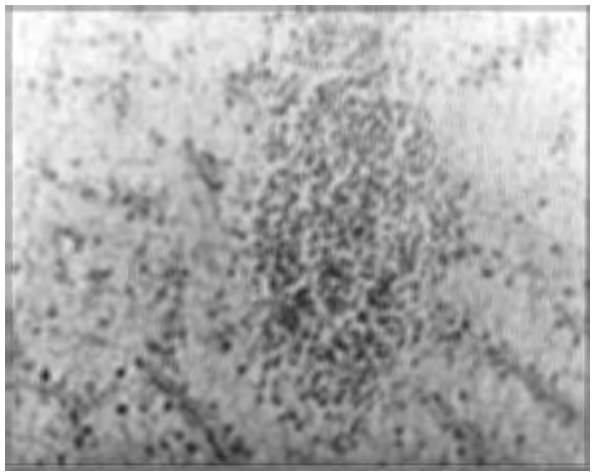

Plate 2. Section of liver showing focal accumulation of leukocytes in the hepatic lobules of fish fed diets (H & E x 400).

Proximate carcass composition of the initial fish and fish sample at end of experiment is shown in Table 5. The highest carcass protein (15.46%) content was obtained in control diet followed by diets 2, 3, 4 and 5 duckweed. In general, there was a decrease in carcass protein and lipid content with the increase of duckweed levels.

Table 5. Proximate carcass composition of the fish at the start and at the end of the experiment (% fresh matter basis)

Parameters	Initial	Final diets				
		1	2	3	4	5
Moisture	78.46	74.17	75.56	75.99	76.84	77.12
Crude protein	14.06	15.46	15.43	15.02	14.56	14.40
Lipid	4.88	6.18	5.48	4.65	3.90	3.68
Ash	2.66	3.31	3.21	3.43	3.69	3.87

Discussion

In the present study, control diet and diet containing 17.07% duckweed showed better performance in term of growth, food conversion and protein utilization in comparison to other diets. Diets containing 34.14%, 51.21% and 59.24% duckweed resulted poor growth performance. The poor growth performances are also substantiated by the histological examination where kidney and liver of fish fed diet containing higher levels of duckweed revealed degenerative changes in the liver.

The findings of the present study indicate that duckweed is comparatively less successful in replacing fish meal protein in comparison to other plant protein sources. Hossain *et al.* (1994) reported that up to 50% of the fish meal protein in *P. gonionotus* diet could be replaced by plant proteins (mustard oilcake and sesame meal) without affecting the growth performance.

In contrast to the present findings, Okoye and Mbagwu (1985) observed higher FCR i.e. poor growth performance of *Sarotherodon galilaeus* fingerling fed diet containing 33% crude protein with 10% duckweed. Attempts by Devaraj *et al.* (1981) to incorporate 40% *Lemna* powder with ricebran, oilcake and ragiflour in common carp diet resulted similarly poor growth and food utilization.

The reason for comparatively better growth of *B. gonionotus* fed on diet containing 17.07% duckweed may be due to the combination of plant protein and animal protein which gave a good nutritional balance. A mixture of plant and animal proteins is much more efficient than that of a single source of protein (Cho *et al.* 1974, Meske and Pruss 1977). Hossain and Jauncey (1990) also reported that use of different protein sources in combinations can prevent a high inclusion level of any single anti-nutritional factor in the diet and can also be a means of compensating for essential amino acid deficiency in any single protein source.

The significantly lower growth of *B. gonionotus* observed with diet containing 34.14%, 51.21% and 59.24% 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 antinutritional 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).

In this study, the apparent protein digestibility (APD) decreased with the higher level of duckweed inclusion. Plant protein appears to be basically less digestible than animal protein (Singh and Pandey 1980). The APD values obtained from this study ranged from 80.10 to 90.42% (Table 4). The fishmeal based control diet in the present study produced the higher APD value (90.42). According to NRC (1977) carp can digest up to 95% of fish meal protein. However, the value may be decreased to 80-85%, depending on the origin and processing of fish meal (Ogino and Chen 1973). The lower APD values obtained in the diets containing duckweed may be due to low digestibility of

plant protein. Hasan *et al.* (1990) also reported lower digestibility of diets with increasing level of dietary water hyacinth and leucaena meal in *Labeo rohita.*

From the histological evidences in the livers of the fish fed higher levels of duckweed it is assumed that an unidentified toxic component in the higher levels of duckweed is responsible for the degenerative changes.

In the present study, the diets containing higher level of duckweed inclusion produced significantly ($P<0.05$) the highest carcass moisture and lower lipid content (Table 5). Hassan and Edwards (1991) reported that feeding duckweed to tilapia had a profound effect on carcass composition i.e. increase in carcass moisture and decrease in carcass lipid content. Similar results have been reported with higher level of dietary plant protein inclusions in common carp (Hossain and Jauncey 1989) and rainbow trout (Yurkowski *et al.* 1978).

Therefore, considering the findings of this study and the availability, cost and abundance of duckweed in Bangladesh, it may be used as an alternative protein source for silver barb but the inclusion level should not exceed 20% duckweed on dry matter basis.

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