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The Effects Of Partially Replacing Fishmeal with Azolla (*Azolla Sp.*) On Growth Parameters Of Shabbout Fish (*Tor grypus* H. 1843)

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Abstract
In this study, the effect of replacement of fishmeal by azolla meal in prepared diet was investigated in Shabbout fish fingerlings. Four different experimental diets (all containing mean 41.7% crude protein and 3026.2 digestible energy) at a various ratios of azolla has prepared. Control diet (D_1) was composed of fishmeal completely. Experimental diets (D_2, D_3 and D_4) were composed of 10%, 20% and 30% azolla meal respectively instead of fishmeal. The experiment was carried out in a closed rearing system with recirculated filtered water for 12 weeks. According the to the results of the experiment, the mean specific growth rate (SGR) and mean weight gain values were the highest for the group fed with D_2 after D_1. There was no difference among four groups with respect to the mean final condition factor (K), feed conversion ratio (FCR) and protein efficiency ratio (PER) values at $P>0.05$ statistical significance level. However, mean weight gain and mean final weight were no significantly different among the groups fed with D_2, D_3 and D_4 ($P<0.05$). SGR and mean final length were significantly different in all groups. Based on all measured criteria, it is suggested that approximately 10% of fish protein from fishmeal can be replaced by azolla meal without adverse effect on fish performance.
Key Word: Shabbout, *Tor grypus*, Feeding, Azolla, Growth

INTRODUCTION

The increasing price of fishmeal and the uncertainty of its procuring lead feed manufacturers and experts in fish feeding to use cheaper and more easily obtainable vegetable protein sources instead of fishmeal protein Alceste [2].

Depending on high protein content and balanced amino acid profile, fishmeal is the main protein source in commercial fish feeds. Due to increasing demand and its use in various animal feed, the shortage in the production of fishmeal in the world doubled. It is clear that developing countries cannot depend on only fishmeal in feeds in the long run. For this reason, various measures have been taken to partially or totally replace fishmeal with other protein sources El-Sayed [8]

Crude protein content of commercial aquaculture feed used in growing fish is 25-45%. The protein of azolla plant having 23-30 % crude protein content includes 55% of the amino acids in present. Water rates are very high and dry matter content ranges from 6 -10%. It is used as pork, chicken, duck, beef and human food Hove [10]. Because of these properties, herbal fish feeds like azolla with high protein content can be used for fish feeds.

Nevertheless research on the use of azolla as a fish feed plant is very limited. In a study on grass carp (*Ctenopharyngodon idella*), it was reported that small water plants such as azolla and lemna were preferred more than others Edwards [7]. It was proved that carps prefered *Azolla carolina* Duthu and Kilgen [6], *tilapia mossambicas* preferred primarily azolla and lemna Lahser [12]. Fiogbe et al. [9] reported that weight gain was observed at all levels *on Oreochromis niloticus* fed with diets supplemented different levels of dry azolla meal (up to 45%). Therefore, the authors stated that azolla could be utilized as a fish feed. Abioye et al. [1] researched the effects of diets supplemented different levels of azolla (up to 100%) instead of palm kernel cake on specific growth rate (SGR), the feed conversion ratio (FCR) and mean weight gain (%) of *Oreochromis niloticus.* The authors showed that there were no significant differences among findings.

Shabbout fish (*Tor grypus*) living in the Tigris and Euphrates rivers is likely to have high commercial value for breeding inland water instead of carp and trout. On the other hand, azolla is not only a cheap source of feeding but also has high protein content. Considering this potential, the present study was designed to determine the effects of supplementing azolla instead of fishmeal in different proportions on growth parameters of shabbout fish.

MATERIALS AND METHODS

Experimental fish

shabbout (*Tor grypus*) fingerlings from Atatürk Dam of the General Directorate of State Hydraulic Works were brought to the laboratory of the Department of Fisheries and Aquaculture of the Faculty of Agriculture at the University of Ankara.

Experimental facilities and procedures

The experiment was conducted in 150-lconical fiberglass tanks containing 50 l of water for 12 week 84 days. An aquaponic system Rakocy et al. [14] integrating fish culture and plant production was used. The plants (tomatoes), embedded in a gravel filter, extract organic wastes from the water and the purified water was recycled back to the fish tanks at a daily exchange rate of 5% (1-1.5 l/min) of the tank volume. *Nitrosomonas* and *nitrobacter* bacteria were added to the gravel beds to enhance the decomposition of nitrogenous compounds. Three replicates

of 10 fish per tank were established for each treatment (Table 1). The average individual weight of the fingerlings was about 4.2 g at the beginning of the experiment. The length and weight of all fish were measured every two weeks. Fish were anesthetized with 0.05 ml/l Quinaldine (Merck Schuchard, FRG) before weighing. Fish were fed *ad libitum* three times per day with one of four experimental diets based on anchovy fishmeal as the sole protein source (Table 2). Feeding table was used in per tank. Proximate analyses of moisture, crude protein (N x 6.25), lipid, and ash of the feed were determined in triplicate by standard methods (AOAC [3]. Water quality, tested weekly according to APHA [4], was 0.084±0.002 ppm total ammonia (NH3-N), 0.084±0.002 ppm nitrite (NO2-N), 230±8.56 total alcalinite, 7.09±0.0097 pH and 5.79±0.065 oxygen. The water temperature was maintained at 26±1 ^{0}C.

Measurements and calculations

At the end of the experiment, growth performance, body composition, and food utilization were calculated as follows: specific growth rate (SGR,%/day)=([lnWt-lnWi]/T) x 100; protein efficiency ratio (PER)=(Wt-Wi)/crude protein fed; feed conversion ratio (FCR)=(CxT)/(Wt-Wi); and condition factor (K)=100x(wt/length3), where W=weight, Wi=initial weight of fish, Wt=final weight of fish, C=daily food intake, and T=duration.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with the SAS package. Duncan's multiple-range test was used to compare differences among individual means. Treatment effects were considered significant at $p<0.05$. Percentage and ratio data were transformed to arcsine values prior to the analysis Zar [18].

Table 1. Layout of the experiment

Test Diets	Relapses	Sources of protein in feeds %		Trial Periods (week)
		Fish meal	Azolla meal	
D_1 (control)	1.1	100	-	12
	1.2	100	-	12
	1.3	100	-	12
D_2	2.1	90	10	12
	2.2	90	10	12
	2.3	90	10	12
D_3	3.1	80	20	12
	3.2	80	20	12
	3.3	80	20	12
D_4	4.1	70	30	12
	4.2	70	30	12
	4.3	70	30	12

Table 2. Composition and proximate analysis of experimental diets (% of raw material).

Diets	D_1 (control)	D_2	D_3	D_4
Ingredient				
Oat	25	15.5	3	-
Soybean oil cake	-	2	5	4
Fishmeal (anchovy)	56	50	44.5	40
Azolla	-	15.5	31	46
vegetable oil	6	6.5	6.5	6
Salt	0.5	0.5	0.5	0.5
Bentonite	3.5	3.5	2.5	0.3
Cornstarch	3	1	2	-
Vitamin mix[1]	1	1	1	1
Mineral mix[2]	1	1	1	1
Methionine	0.5	0.5	0.5	0.5
Gelatin	3.5	3	2.5	0.7
Proximate analysis				
Dry matter (% of air-dry wt)	93.7	94.0	93.9	93.8
Crude protein	42.5	41.9	41.5	40.7
Lipid	12	9.6	8.8	8.3
Ash	12	15	17.7	19.2
Crude fiber	2.55	2	3.6	5
Metabolizable Energy(Kcal/kg)	3210	3040	2945	2910

[1]Rovimix 123-T25K (per 2.5 kg): Vitamin A 12,000,000 UI; vitamin D3 2,000,000 UI; vitamin E 35,000 mg; vitamin K3 4,000 mg; vitamin B1 3,000 mg; vitamin B2 7,000 mg; vitamin B6 5,000 mg; vitamin B12 15 mg; vitamin C 50,000 mg; niacin 20,000 mg; folic acid 1,000 mg; calcium Dpantothenate 10,000 mg; biotin 45 mg; choline chloride 125,000 mg.
[2]Remineral S 25K (per kg): Fe 60,000 mg; Cu 5000 mg; Mn 80,000 mg; Co 200 mg; Zn 60,000 mg; I 1,000 mg; Se 150 mg.

Table 3. Growth performance, feed conversion, and protein efficiency of shabbout fish (*Tor grypus* H. 1843*)* fingerlings fed diets containing different rates azolla meal instead of fish meal for 12 weeks.

	Test Diets (fish meal protein/azolla meal protein)			
	D_1 (100/0) control	D_2 (90/10)	D_3 (80/20)	D_4 (70/30)
Mean initial wt (g)*	4,2±0,263[a]	4,23±0,209[a]	4,22±0,231[a]	4,21±0,359[a]
Mean final wt (g)*	14,4±0.834[a]	11,7±0.634[b]	10,02±0.513[b]	10,59±0.600[b]
Mean initial length (mm)*	7,66±0,186[a]	7,77±0,143[a]	7,84±0,172[a]	7,66±0,200[a]
Mean final length (mm)*	11,95±0.252[a]	11,29±0.207[b]	10,61±0.192[c]	10,88±0.235[bc]
Mean wt gain (%)*	29.5±1.64[a]	24.8±0.466[b]	21.7±0.252[b]	22.9±0.673[b]
Mean initial condition factor (K)*	0,91±0,02[a]	0,89±0,02[b]	0,86±0,02[b]	0,87±0,019[b]
Mean final condition factor (K)*	0.82±0.016[a]	0.79±0.010[a]	0.82±0.011[a]	0.81±0.0221[a]
Food conversion ratio (FCR)[1*]	2.44±0.234[a]	3.28±0.198[a]	2.93±0.464[a]	3.43±0.222[a]
Specific growth rate (SGR; %/day)*	1,36±0.0842[a]	1,10±0.0266[b]	1,03±0.0152[c]	1,05±0.0396[bc]
Protein efficiency rate (PER)[1*]	1,50±0.08[a]	2,06±0.184[a]	1,57±0.07[a]	1,91±0.204[a]
Survival (%)	100	100	100	100

Values with different superscripts differ significantly ($p<0.05$).
[1]Expressed as the percent of the initial body weight after 12 weeks.
[2]Moisture-free basis.

RESULTS

The final average weight, mean weight gain of fish fed with D_1 were significantly higher than those of fish fed with D_2, D_3 and D_4. Final condition factor (K), the feed conversion ratio (FCR) and protein efficiency rate (PER) did not significantly differ among the groups. Specific growth rate (SGR) significantly differ among the groups. Survival was high for all treatments (Table 3).

DISCUSSION

We could not find any research on supplementing only azolla instead of fishmeal in the course of conducting our study. Therefore, researches about other sources of plant protein could shed light on the comparison of our findings. By taking into consideration, we compared our findings with values obtained from the studies on diets replacement of fishmeal with soybean.

According to our study, the highest average live weight as a result of a 12 week experiment was observed in group fed with D_1 (14.4±0834) and the lowest one with D_3 (10.02±0513). The average live weight gain (%) at the end of the trial was 29.5±1.64%, 24.8±0466%, 21.7±0252%, 22.9±0673% in groups fed with D_1, D_3 and D_4 respectively. The difference was significant in the group fed with between D_1 and other diets (D_2, D_3, D_4) ($p<0.05$). But there was no significant difference among groups fed with D_2, D_3, D_4 ($p>0.05$).

The results obtained from the studies on diets replacement of fishmeal with soybean are important for evaluating the results of our study. Tuladhar [17] reported that total yield in group fed with azolla, soybean and oil cake increased 38.79% compared with fish meal in carp polyculture systems over a period of 8 months. According to 42-day feeding study, 1.43 kg fishmeal was necessary, while 0,716 kg of azolla, soybean and oil cake diet was enough to produce 1 kg of fish. In short, FCR in that group was found higher than the group fed with fishmeal. The yield in groups fed with diets consist of azolla, soya beans and oilcakes was higher compared to the groups fed with diets consist of fishmeal. However, in our study, in terms of FCR, there was no significant difference among all groups ($p>0.05$). In our opinion, this difference is due to the difference in fish species and the breeding system used.

Sudaryono [16] observed the effects of replacement of soybean meal by azolla meal on growth parameters of *Penoeus monodon* fry. The research indicated that there was no difference in diets supplemented azolla instead of soybean meal (100 %) in terms of weight gain, growth rate, SGR, feed intake, survival, PER and APU ($P<0.05$). In other words, the study showed that the effects of diets with azolla was similar to the effects of diets with soybean.

Chou et al. [5] researched on the effects of soybean instead of fishmeal up to 60% in cobia fry (Rachycentron). They determined that FCR significantly increased while weight gains, PER and NPU (net protein utilization) decreased depending on the increment in the share of soybean meal. Up to 40% uplementation of soybean, there was no differences among diets in terms of FCR. These results are parallel to FCR values in our study. In addition to this, the study by Chou et al. [5] the optimum soybean supplementation was reported as 16.9 %.

In another study, four diets containing 30% crude protein were tested on channel catfish juveniles. In these diets, 30%, 20%, 10% and 0% fishmeal was replaced with 10%, 20%, 30% and 40% soybean meal respectively. The study suggested up to 10% fishmeal replacement by soybean meal without change in the growth of juveniles Rab et al. [13]. Our study conducted with azolla also reveals similar results. On the other hand, the replacement percentage between fishmeal and soybean that led to reduce the growth was reported over 20% in yellowtail by Shimeno et al. [15] and 25% in rainbow trout and Atlantic salmon by Kaushik et al. [11].

CONCLUSION

The results of the present study indicate that azolla is a source of plant protein that can be used in fish culture. Based on all measured criteria, it is suggested that 10 % of fish protein from fishmeal can be replaced by Azolla. Furthermore, incorporation of azolla will provide relatively low cost fish diets.

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