EFFECT OF STOCKING DENSITY ON MUGILL FISH (*Mugill cephalus*) PERFORMANCE UNDER DIFFERENT LEVELS OF AZOLLA MEAL

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SUMMARY

Two feeding trials were conducted at Wady El-Natroun, El- Behera Governorate, Egypt to determine the effect of feeding *Azolla pinnata* meal as partial replacement of the total protein in *Mugill cephalus* diets. Fish with average initial body weight of approximately 71.55g were randomly allocated to four dietary treatments at two stocking densities (1000 fish/pond and 1500 fish /pond), two replicates per treatment. Dietary treatments were control diet without Azolla meal, control diet with 10 % Azolla meal, control diet with 20% Azolla meal and control diet with 30% Azolla meal as replacements from total protein diets. Fishes were fed pelleted compounded feed (32 % CP and 4439 kcal gross energy/Kg DM of diet) twice daily at ratio 3% of their weights during the experimental period (120 days) and the fishes were slaughtered for carcass evaluation at the end of the experimental period. The results revealed that, water *p*H and temperature were not significantly different among experimental groups whereas, lowest values of NH3 concentrations were observed with control and 20% Azolla diet. The highest dissolved oxygen was observed with inclusion of Azolla meal in diets at the first stocking density (1000 fish/pond) and with 20 and 30% Azolla inclusion diet at the second stocking density (1500 fish/pond). Average daily gain and specific growth rate (SGR) were significantly improved with 20% Azolla meal at the two densities. Condition factor (CF) was significantly ($P<0.05$) lowest in the diet with 20% Azolla meal at the second density (1500 fish/pond) but the difference was insignificant at the first (1000 fish/pond) density. Daily feed intake (DFI) and feed conversion ratio (FCR) of fishes at the two densities were not significantly different among experimental groups. The edible parts (%), nonedible parts (%) and fillet dressing (%) were similar for all treatments in two densities.

Keywords: Azolla meal, Mugill fish, stocking density, growth performance, water quality.

INTRODUCTION

Protein is the most important component of the diet of fish because protein intake generally determines growth has a high cost per unit and high levels are required per unit of feeds.

Whereas, many fish species have a high dietary protein requirement (30 to 50%), which vary for each species and with each particular life stage. Because of the limited supplies and the high price of fishmeal, other alternative sources of protein must be considered.

Plant proteins are generally cheaper per unit of nutrient than animal protein. Azolla is widely distributed and is found in tropics and sub-tropics and It grows naturally in stagnant water of drains, canals, ponds, rivers, marshy lands with a temperature range of 14 - 40°C (Singh and Subudhy 1978; Khan, 1988). Furthermore, Azolla is a potential source of nitrogen and is a potential feed ingredient (Lumpkin, 1984; Pannerker, 1988) whereas; Azolla contains around 27% crude protein, 10% carbohydrates on a dry weight basis and lower ash (10.5%) content (Pullin and Almazan 1983).

However, high levels of plant proteins in fish diets have in some cases resulted in reduced growth and poor feed efficiency, probably the result of improper balance of essential nutrients, such as amino acids and minerals, presence of toxic substances or antinutritional factors, or decrease of palatability and pellet water stability value (Lim and Dominy ,1991).

With those considerations, nutritional studies were carried out to investigate the performances of Mugill fishes (*Mugill cephalus*) reared under two stocking densities with containing different levels of Azolla diets.

MATERIALS AND METHODS

Preparation and analysis of the experimental feeds:

Azolla pinnata was collected from the main canals in Abbassa, Sharkeia Governorate, Egypt and washed with tap water to remove contaminants, like snails and soil particles and then sun dried for about three days. It was ground in a Wiley mill to a fine powder and incorporated into the experimental diets. Sample preparation and analyses of the dietary ingredients for dry matter (DM), crude protein (CP), crude fiber (CF), crude fat and ash (Table 1) were determined according to A.O.A. C (1990).

Culture conditions

The feeding trial was conducted in 16 stagnant- water earthen ponds, each about 1000 m2 which located at Wady El- Natroun, El- Behera Governorate, Egypt. These 16 ponds were allocated to eight treatments with two replications per treatment. The average initial body weight of the Mugill fish (*Mugill cephalus*) in each treatment at start was approximately the same (71.55g).

Fishes were fed pelleted compounded feed (32 % CP and 4439 kcal gross energy/Kg DM of diet) twice a day at a daily ratio of 3% of their body weights. Four diets were formulated in which 0, 10, 20, 30% of

the basal diets (control) were replaced by Azolla meal (Table 1). Two socking densities were used, the first was 1000 fish/pond and the second was 1500 fish /pond.

To determine growth in weight (g/fish) and length (cm/fish) and the amount of feed required of fishes, a sample of about 50 fish /pond were taken every month throughout the experiment. At the end of the experiment (120 days), the fishes were weighed and measured individually and five fishes per pond were selected at random for carcass analysis. The fishes were slaughtered and the carcass traits and composition were recorded.

During the experiment, several water quality parameters were measured and these were water temperature, pH, dissolved oxygen (DO), ammonia and Secchi disk (S.D) monitored for each pond at regular intervals following the procedure recommended by the APHA (1985).

Calculation:

Gross energy estimated (5.65, 9.4, 4.0, and 4.0 cal GE / g Dry matter for CP, EE, CF and NEF, respectively according to Jobilling (1983).

Total gain (%) = Wt (final)-Wt (initial)/ Wt (initial)*100

FCR= Feed intake (g)/ [Wt (final, g)-Wt (initial, g)]

PER= [Wt (final, g)-Wt (initial, g)] /protein intake (g)

Condition factor (CF) = (W/L^3) *100.

SGR = [(In final weight–In initial weight)/period] *100.

Statistical analysis:

Data were analyzed using one way ANOVA procedure of SAS (١٩٩٩). Differences among means were compared using Duncan's Multiple Range Test (Duncan, ١٩٥٥).

RESULTS AND DISCUSSION

During the culture period, at the two stocking densities, water pH and temperatures were not significantly different among experimental groups (Table 2) and pH was optimum and within the values (6.5 to 9) recorded by Woynarovich, (1981).

Fish fed the control and 20% azolla diets resulted in the lowest NH3 concentrations. The highest dissolved oxygen was observed for the first stocking density (1000 fish/pond) in groups fed 20 % and 30 % Azolla diets and with the second stocking density (1500 fish/pond) in group fed 30% azolla diet. Whereas, dissolved oxygen is one of the most imported factors affecting growth and causing mortality whereas, concentration of 1.4 mg/l oxygen is sufficient to maintain life in water (George, 1961) and the optimal range of dissolved oxygen is between 5 and 8 mg/l (Jhingran, 1991). This might be due to relative reduction of respiratory of fish with low stocking density.

The growth response of Mugill fish fed diets with different levels of Azolla meals at two varying densities are shown in Table (3).

Table (1): Composition and chemical analysis (% DM basis) of the feed ingredients and the experimental diets used in the feeding experiments.

Ingredients	Experimental diets (%)			
	Control	10	20	30
Fish meal	25	25	25	25
Soybean meal	16.35	13.15	10.15	6.85
Azolla meal	0	10	20	30
Wheat bran	33.90	28.89	22.70	18.90
Yellow corn	18.25	16.46	15.65	12.75
Soybean oil	3	3	3	3
Vit. Min. mixture	2	2	2	2
Dicalcium phosphate	1.5	1.5	1.5	1.5

Feed ingredients	Chemical composition on dry matter basis (%)					
	DM[1]	CP[2]	CF[3]	EE[4]	Ash	NFE[5]
Herring fish meal	92.70	70.90	0.85	9.74	14.27	4.24
Soybean meal	90.13	46.55	6.59	1.78	7029	37.79
Yellow corn	89.04	10.58	3.65	4.55	1.59	79.63
Wheat bran	88.81	13.95	8.13	2.85	3.98	71.09
Azolla meal	90.26	23.91	14.89	3.19	24.88	33.13
Experimental diet						
Zero Azolla	92.58	31.96	4.20	6.45	9.44	47.95
10% Azolla	92.38	32.04	5.43	6.85	10.04	45.64
20% Azolla	90.16	32.11	5.91	6.96	11.58	43.44
30% Azolla	91.86	32.19	6.92	7.92	13.09	39.88

1-Dry matter, 2-Crude protein, 3-Crude fiber, 4-Ether extracts, 5-Nitrogen free extract

Table (2): Water quality with Mugill fish fed different percentages of Azolla meal in diets at the end of experimental period (Mean ± SE*).

Water quality parameters	Mugill 1000 fish / pond				Mugill 1500 fish / pond			
	Air dried Azolla levels				Air dried Azolla levels			
	zero	10%	20%	30%	zero	10%	20%	30%
pH	8.50^{a} ±1.33	8.30^{a} ±1.33	8.60^{a} ±1.33	8.70^{a} ±1.33	8.40^{a} ±1.33	8.30^{a} ±1.33	8.70^{a} ±1.33	8.80^{a} ±1.33
Temperature	30.7^{a} ±2.21	30.8^{a} ±2.21	30.9^{a} ±2.21	31.1^{a} ±2.21	30.6^{a} ±2.21	30.6^{a} ±2.21	31.2^{a} ±2.21	30.9^{a} ±2.21
Dissolved oxygen (mg /l)	4.8^{b} ±0.70	5.1^{a} ±0.70	5.1^{a} ±0.70	5.2^{a} ±0.70	4.4^{c} ±0.70	4.4^{c} ±0.70	4.8^{b} ±0.70	4.9^{b} ±0.70
Ammonia (NH_3 mg/L)	0.17^{b} ±0.01	0.18^{ab} ±0.01	0.17^{b} ±0.01	0.21^{a} ±0.01	0.18^{b} ±0.01	0.20^{ab} ±0.01	0.19^{ab} ±0.01	0.23^{a} ±0.01
Secchi disk (S.D) (cm)	18.5^{a} ±0.02	16.3^{a} ±0.02	17.2^{a} ±0.02	12.8^{b} ±0.02	10.3^{c} ±0.02	13.3^{b} ±0.02	13.0^{b} ±0.02	11.4^{c} ±0.02

a, b and c in the same row are significantly different ($P<0.05$).

* Standard error of means

Fish fed the Azolla diets of 20% showed the best average final body weight (AV. FBW) and average weight gain (AV. TWG) compared to the fish fed the other diets with significantly difference ($P<0.05$) at the two tested densities.

The average daily gain of experimental fish showed that fish fed on 20% Azolla diet at the two densities had significantly higher values than those fed on other diets. Similar results were obtained by Santiago *et al.* (1987) who found that the growth of Nile tilapia increased and feed conversion improved as the level of the dietary Azolla meal increased.

Moreover, specific growth rate (SGR) values obtained for fish fed diets with different levels of incorporation of Azolla at the two densities were not significantly different from those fed the control diet. The lowest condition factor (CF) observed with fish fed diet 20% Azolla with no significant difference at the first stocking density (1000 fish/pond) density, however the differences were significantly ($P<0.05$) at the second density (1500 fish/pond).

In feeding trials with the experimental feeds (Table 4), results revealed that there were no significant differences among experimental groups at the two density of fish daily feed intake (DFI) and feed intake of crude protein (CPI), feed conversion ratio (FCR), feed efficiency (FE). Protein efficiency ratios (PER) for fish fed control and 10% Azolla diets were significantly ($P<0.05$) higher than those fed 20% and 30% Azolla diets at the first density (1000 fish/pond), however at the second density (1500 fish/pond), fish fed diets control, 20% and 30% Azolla had significantly higher values of PER compared to fish fed 10% Azolla diet. Whereas, Alcantara and Querubin (1985) concluded that nutrient digestibilities of crude protein, crude fat, and crude fiber were not affected by the level of Azolla in the ration, and that broilers can readily digest the crude fiber in Azolla, so that digestibility may not have been a limiting factor.

Carcass traits and chemical composition of Mugill fish at the end of the feeding experiment are presented in Table (5). The edible parts (%), nonedible parts and fillet dressing (%) were not affected by dietary treatment. However, viscera content (%) in the finally sampled fish was significantly higher ($P<0.05$) than in the control at the first density (1000 fish/pond) whereas, no significant differences were obtained between the fish fed the diets with different Azolla inclusion. However, there were no significant differences in viscera content between control diet and 10% Azolla containing diets, also between 20% and 30% Azolla containing diets.

The protein content of fish fed different diets at the first density (1000 fish/pond) did not differ significantly from the control, whereas protein content obtained for fish fed on 20% Azolla diet were significantly ($P<0.05$) higher than that obtained for fish on different Azolla diets at the second density (1500 fish/pond). The lowest values of fat content were obtained with fish fed on 20% and 30% Azolla diets. However, the difference were significant ($P<0.05$) as compared to fish fed the control and 10% Azolla diets at the first density. Similar results were

Table (3): Growth performance and condition factor of Mugill fish fed different levels of Azolla meal at two densities (Mean ± SE*).

Items**	Mugill 1000 fish / pond				Mugill 1500 fish / pond			
	Air dried Azolla levels				Air dried Azolla levels			
	zero	10%	20%	30%	zero	10%	20%	30%
Initial BW (g)	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61	71.55^{a} ±0.61
Final BW (g)	319.08c^{d} ±2.70	329.70^{b} ±2.70	346.11^{a} ±2.70	330.17^{b} ±2.70	323.07^{c} ±2.70	315.11^{d} ±2.70	340.18^{a} ±2.70	331.30^{b} ±2.70
TWG (g)	247.53^{c} ±3.39	258.15^{b} ±3.39	274.56^{a} ±3.39	258.62^{b} ±3.39	251.52^{c} ±1.16	243.56^{d} ±1.16	268.63^{a} ±1.16	259.75^{b} ±1.16
DG (g)	2.06^{b} ±0.06	2.15^{ab} ±0.06	2.29^{a} ±0.06	2.16^{ab} ±0.06	2.10^{ab} ±0.06	2.03^{b} ±0.06	2.24^{a} ±0.06	2.16^{ab} ±0.06
WG%	345.95^{c} ±0.73	360.40^{b} ±0.73	383.73^{a} ±0.73	361.45^{b} ±0.73	351.53^{c} ±0.48	340.40^{d} ±0.48	375.44^{a} ±0.48	363.03^{b} ±0.48
SGR	1.25^{a} ±0.07	1.27^{a} ±0.07	1.31^{a} ±0.07	1.27^{a} ±0.07	1.26^{a} ±0.03	1.24^{a} ±0.03	1.30^{a} ±0.03	1.28^{a} ±0.03
C.F	1.36^{a} ±0.03	1.40^{a} ±0.03	1.35^{a} ±0.03	1.39^{a} ±0.03	1.33^{b} ±0.02	1.37^{ab} ±0.02	1.32^{b} ±0.02	1.41^{a} ±0.02

a,b,c and d in the same row are significantly different (P<0.05). *SE means standard error of means

**AV. FBW= average final body weight, BW =body weight (g), AV. TWG = average total weight gain, SGR= specific growth rate, C.F =condition factor

Table (4): Feed utilization of Mugill fish fed diets with different levels of Azolla meals (Mean±SE*).

Item**	Mugill 1000 fish / pond				Mugill 1500 fish / pond			
	Air dried Azolla levels				Air dried Azolla levels			
	zero	10%	20%	30%	zero	10%	20%	30%
FI (g/d)	3.73^{a} +0.25	3.96^{a} ±0.25	4.41^{a} ±0.25	4.21^{a} ±0.25	3.89^{a} ±0.14	3.97^{a} ±0.14	4.31^{a} ±0.14	4.12^{a} ±0.14
CPI (g/d)	1.18^{a} +0.08	1.27^{a} ±0.08	1.42^{a} +0.08	1.36^{a} ±0.08	1.24^{a} ±0.09	1.27^{a} ±0.09	1.38^{a} ±0.09	1.33^{a} ±0.09
FCR	1.81^{a} ±0.06	1.84^{a} ±0.06	1.93^{a} ±0.06	1.95^{a} ±0.06	1.86^{a} ±0.05	1.96^{a} ±0.05	1.92^{a} ±0.05	1.91^{a} ±0.05
PER	1.73^{a} ±0.03	1.69^{ab} ±0.03	1.61b^{c} ±0.03	1.59^{c} ±0.03	1.69^{a} ±0.02	1.60^{b} ±0.02	1.62^{ab} ±0.02	1.62^{ab} ±0.02

a, b and c in the same row are significantly different (P<0.05). *SE means standard error of means

**FI= daily feed intake, CPI= feed intake of crude protein, FCR= feed conversion ratio, FE= feed efficiency PER= protein efficiency ratios

Table (5): Carcass traits and carcass composition of Mugill fish fed the experimental diets (Mean±SE*).

Item	Mugill 1000 fish / pond				Mugill 1500 fish / pond			
	Air dried Azolla levels				Air dried Azolla levels			
	zero	10%	20%	30%	zero	10%	20%	30%
Edible parts (%)	61.10^{a} ±o.47	60.85^{a} ±o.47	61.34^{a} ±o.47	60.64^{a} ±o.47	61.26^{a} ±0.64	60.71^{a} ±0.64	59.85^{a} ±0.64	61.57^{a} ±0.64
Nonedible parts (%)	38.90^{a} ±0.43	39.15^{a} ±0.43	38.66^{a} ±0.43	39.36^{a} ±0.43	38.74^{a} ±0.60	39.29^{a} ±0.60	40.15^{a} ±0.60	38.43^{a} ±0.60
Fillet Dressing (%)	39.00^{a} ±1.11	36.88^{a} ±1.11	38.93^{a} ±1.11	36.66^{a} ±1.11	39.10^{a} ±0.34	39.0^{a} ±0.34	38.64^{a} ±0.34	39.01^{a} ±0.34
Viscera content (%)	8.0^{b} ±0.32	9.17^{a} ±0.32	9.10^{a} ±0.32	9.40^{a} ±0.32	10.95^{a} ±0.42	11.20^{a} ±0.42	9.80^{bc} ±0.42	9.22^{c} ±0.42
Chemical composition (on DM basis %)								
DM[1]	26.13^{c} ±0.24	27.33^{b} ±0.24	28.81^{a} ±0.24	28.95^{a} ±0.24	25.60^{c} ±0.32	27.00^{b} ±0.32	28.13^{a} ±0.32	28.66^{a} ±0.32
CP[2]	61.85^{b} ±0.31	63.50^{a} ±0.31	64.11^{a} ±0.31	63.76^{a} ±0.31	62.07^{b} ±0.30	61.80^{bc} ±0.30	63.15^{a} ±0.30	61.03^{c} ±0.30
FAT	22.51^{b} ±0.38	23.71^{a} ±o.38	20.83^{c} ±0.38	20.33^{c} ±0.38	22.70^{ab} ±0.45	23.66^{a} ±0.45	22.40^{ab} ±0.45	22.15^{b} ±0.45

a, b and c in the same row are significantly different (P<0.05). *SE means standard error of means

1=dry matter 2=crude protein

obtained by Micha *et al.*, (1988) who found that fresh Azolla in the diets for *O. niloticus* and *T. rendalli* fingerlings reduced drastically the lipid content of body tissues for both species of fish whereas crude protein content was not affected.

Generally, the present study agrees with Santiago *et al.* (1987) who reported that Azolla should not absolutely be rejected from fish culture diet and Shaker *et al.* (2000) who reported that growth performance parameters up to 20% Azolla can replace fish diet containing fish meal, whereas, on body composition of fish, up to 10% Azolla are recommended.

CONCLUSIOIN

Results of these trial indicated that Azolla can partially replace dietary protein up to a level of about 20% of the total crude protein in mugill fish diets under tow stocking densities (1000 and 1500 fish\pond) without any problems and with no adverse effects on growth rate. Thus it can be concluded that azolla can effectively replace up to 20 % of the normal level of total crude protein with no reduction in growth.

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تأثير كثافة التخزين على أداء أسماك البورى تحت مستويات مختلفة من الأزولا

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تم إجراء هذه التجربة في وادي النطرون- محافظة البحيرة (مصر) وذلك بهدف دراسة تأثير استخدام مستويات مختلفة من الازولا (0 و 10 و 20و 30% كمصدر بروتين فى علائق اسماك البوري و ذلك تحت كثافتين مختلفين (1000 و 1500 سمكة / حوض).واستخدم فى هذة التجربة عدد 20000 اصبعية بورى بمتوسط وزن ابتدائى (71.55 جرام) وتم تسكين الاسماك في أحواض ترابية بمساحة 1000 م2 / حوض بمعدل حوضيين لكل معاملة و تم تغذية الأسماك بمعدل 3% من وزن الجسم فى اليوم على العلائق التجريبية المتماثلة فى نسبة البروتين و الطاقة (32 % بروتين خام و 4468 ك ك طاقة كلية / كجم مادة جافة) وذلك مرتين يوميا لمدة 120 يوم .

وقد اوضحت النتائج التالى :

1- أنه لم يحدث اختلاف معنوي في كلا من درجة الأس الهيدو جيني و درجة الحرارة بينما تم الحصول على ادني قيم تركيز الامونيا مع علائق الكنترول و 20% ازولا و تم الحصول على اعلي تركيزللاكسجين الذائب مع عليقه 20% و 30% ازولا عند الكثافة الأولى (1000 سمكة / حوض) وكانت مع 30% ازولا عند الكثافة الثانية (1500 سمكة /حوض).

2- تحسن متوسط الوزن النهائي لمجاميع الأسماك التي تغذت على 20% ازولا مقارنة بباقي العلائق وذلك تحت الكثافتين.

3- حدث تحسن معنويا في متوسط الزيادة اليومية و معدل النمو النسبي عند 20% ازولا مقارنة بباقي العلائق تحت الكثافتين.

4- عدم حدوث أى اختلاف معنوي في كلا من المأكول اليومي من المادة الجافة و البروتين الخام وكلا من الكفاءة التحويلية و الكفاءة الغذائية بين العلائق المختلفة تحت الكثافتين.

نستنتج أنه من الممكن استخدام الازولا كمصدر بديل للبروتين بالعلائق حتى 20 % فى علائق اسماك البورى دون اى تاثيرات معنوية على النمو و الاداء ومكونات الجسم وصفات جودة المياه.