Egypt. J. Agric. Res., 76 (3), 1998.

EVALUATION OF *AZOLLA PINNATA* MEAL AS AN INGREDIENT IN DIETS FOR *TILAPIA ZILLII* FRY

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(Manuscript received 30 December 1997)

Abstract

Duplicate groups of *Tilapia zillii* fry reared in ten aquaria were fed one of isonitrogenously five different diets for 90 days. Formulated control diet containing 20% crude protein was used. A whole part of the diet was replaced with either 0, 25, 50, 75 or 100% *Azolla pinnata* meal. Results showed growth retardation of the fish proportional to the increasing Azolla level in the diet. Mortality rate reached 23% for fish fed 100% Azolla meal. Carcass composition showed increased moisture and ash contents and poorer content of CP and EE. Values of feed conversion ratios were higher; PER, PPV% and EU% were lower with increasing *Azolla* level. *Azolla* is not nutritionally efficient for *T.zillii* fry at high inclusion levels. However, levels below 25% may be promising as an ingredient in fish feed in view of being costless protein source.

INTRODUCTION

Azolla, an aquatic floating fern, is widely distributed throughout tropical and subtropical zones. It rapidly propagates and may double its biomass in 3-10 days reaching a standing crop of 8-10 tons/ha in rice fields (Pullin and Almazan 1983). This huge mass of high protein content (Moore 1969, Becking 1979, Peters *et al.* 1979, Chen and Huang 1987) had encouraged testing its use as fish fodder. Feeding tilapias with fresh or dried *Azolla* has received special interest (Angeles 1984, Antoine *et al.* 1986, Pantastico *et al.* 1986, Antoine *et al.* 1987, Micha *et al.* 1988, Santiago *et al.* 1988 and EL-Sayed 1992). However, results were controversial.

The present study was conducted to assess the nutritive value of Azolla meal as an ingredient in *Tilapia zillii* fry diet.

MATERIALS AND METHODS

Fresh *Azolla pinnata* cropped from the draining system of the experimental fish farm of the Central Laboratory for Aquaculture Research (CLAR), were sun-

dried and finely ground, pelleted and analysed for chemical composition. A diet (containing approximately the same protein percentage) was formulated to be used as a control diet and as a base for formulating the other experimental diets. Ingredients of the base diet were 20% fish meal, 33% yellow corn, 33% wheat bran, 7% wheat flour, 2% corn oil, 2.5% vitamin premix and 2.5% mineral premix. A whole part of the base diet was replaced with different levels of Azolla meal. Thus, five tested diets were formulated by mixing either 0.0, 25.0, 75.0 or 100.0% Azolla meal (diets 1 to 5, respectively) with preformulated fish feed.

Tilapia zillii fry were collected from CLAR fish ponds, graded for similar sizes (average 2.29 ± 0.08g) and acclimatized for 15 days to laboratory conditions. Fifty fish were frozen at-20°C for chemical analysis. The fry of mixed sex were distributed randomly at a rate of 15 fish/aquarium (40x50x60cm) with fixed water volume of 50 liters per each. Well aerated water supply was provided from a storage fiberglass tank. Air was supplied from a central aeration system. Settled fish wastes were siphoned daily together with half of the water volume which was replaced from the storage tank. Temperature was approximately kept at 28 ± 1°C. Two aquaria were assigned for each diet, and fish were fed frequently to satiation for 90 days. The experiment was terminated by removing the fish and subjecting them to proximate chemical analysis according to A.O.A.C. (1990). Growth performance was determined. Nutrient utilization was calculated after Zein-Eldin and Meyers (1973). Gross energy of the diet and fish carcasses was estimated according to NRC (1993). Statistical analysis was conducted following Snedecor and Cochran (1971) and Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Chemical analysis of the locally growing *Azolla pinnata* diet 5 (100% Azolla) presented in Table 1 showed low protein content (19.88±0.71%) within the range of 18.8 and 22.2% reported by Almazan *et al.* (1986) and Angeles (1984), respectively, and lower than that range reported by EL-Sayed (1992). The chemical composition of Azolla can be affected by the population density (De Waha Baillonville *et al.* 1984), and, not only the species and ecotypes, but also, with the ecological conditions and the phase of growth (Van Hove *et al.* 1987). *Tilapia zillii* were chosen as the test fish based on the knowledge of being phagocytolytic omnivore fish (Hickling 1971, Spataru 1978 and Balarin and Hatton 1979). Thus, it may accept a plant-based formulated Azolla diet and efficiently utilize it.

Table 1. Composition and proximate chemical analysis (on DM bases) of the experimental diets containing different *Azolla* meal levels.

Item	Diet				
	1	2	3	4	5
Ingredient (%)					
Fish feed	100.0	75.0	50.0	25.0	0.00
Azolla meal	0.00	25.0	50.0	75.0	100.0
Total	100.0	100.0	100.0	100.0	100.0
Chemical analysis (%)					
DM	86.51±0.39	86.31±0.31	87.19±0.27	87.45±0.47	87.38±0.38
CP	20.33±0.73	20.56±0.09	20.43±0.12	20.26±0.15	19.88±0.71
EE	3.58±0.33	3.43±0.44	3.04±0.18	2.05±0.23	2.61±0.34
Ash	19.02±0.01	21.54±0.03	25.10±0.31	25.34±0.94	26.31±0.17
Fibre	9.81±0.16	12.60±0.80	14.90±0.07	18.01±0.12	20.16±0.90
NFE*	47.26	41.87	36.53	33.34	31.04
GE (Kcal/g)**	3.42	3.20	2.94	2.8	2.64

* NFE : Calculated by difference.

** GE : Calculated after NRC (1993) as 5.64, 9.44 and 4.11 Kcal/g for protein, lipid and nitrogen free extract, respectively.

Growth performance of *T.zillii* fry (Table 2) was generally very poor on all the test diets. This may be attributed to the low protein content of these experimental diets (About 20%), while, protein requirement for tilapia fry is 35% (Mazid *et al.* 1979 and DeSilva *et al.* 1989), and may be as mush as 40% (Teshima *et al.* 1978).

Table 2. Growth performance of *Tilapia zillii* fry fed on diets containing different levels of *Azolla* meal.

Diet	Initial weight (g/fish)	Initial weight (g/fish)	Weight gain (g/fish)	SGR (%)	Mortality (%)
1	2.30 ± 0.01	4.72±0.2a*	2.42 ± 0.21a	0.79a	6.67±0.0b
2	2.22 ± 0.01	4.65±0.2a	2.43 ± 0.22a	0.82a	10.00±3.3b
3	2.32 ± 0.09	3.14±0.1c	0.82± 0.08c	0.33c	10.00±3.3b
4	2.35 ± 0.01	2.72±0.0cd	0.37± 0.03cd	0.16cd	16.67±3.3ab
5	2.27 ± 0.03	2.49±0.0d	0.22 ± 0.01d	0.10d	23.34±3.3a

* Figures in the same column not having the same letters are significantly different ($P<0.05$).

Response of *T.zillii* to increasing incorporation level of Azolla meal in their diets was negative, resulting in proportional decrease in final weight gain, specific growth rate (SGR%) and higher mortality rate. The negative role of Azolla on fish growth has been previously reported. Our results are in agreement with Micha *et al.* (1988) who reported a decreased growth of both *Oreochromis niloticus* and *T.rendalli* when Azolla was incorporated in their diets. Moreover, fish (*O.niloticus*) suffered weight loss on dried *Azolla* powder or all *Azolla* pellets. Though positive growth was obtained with pellets containing more than 50% *Azolla*, growth was significantly worse than that obtained with *Azolla*-free diet (Almazan *et al.* 1986). Similar results were obtained with both *O.niloticus* fry and adults fed either fresh or dry *Azolla pinnata* (EL-Sayed 1992). The controversy about *Azolla* as an ingredient in tilapia feeds ranged between complete refusal (Almazan *et al.* 1986) and complete acceptance (Santigao *et al.* 1988). In a recent study by El-Sayed (1992), a diet with 30% *Azolla* level (substituting 25% of fishmeal protein) did not support similar growth as *Azolla*-free diet. The present study showed that, replacement of 25% of fish diet with *Azolla* (substituting 35% of the fishmeal protein) did not significantly ($P>0.05$) reduce growth performance of *T.zillii*. However, fish fed higher levels of *Azolla* meal were exposed to death where mortality rate of the group fed on *Azolla*

only diet was 23% which is four times higher than mortality rate of fish fed on *Azolla*-free diet, while, only 10% mortality rate was reported by Micha *et al.* (1988).

The poor performance of *T.zillii* fed on diets with high levels of *Azolla* meal may be due to its low nutritive value and the deficiency in some essential amino acids especially, methionine, lysine and histidine, as well as, the high neutral detergent fiber of Azolla, and possibly, adenine which limits the usefulness of *Azolla* as a food ingredient for simple-stomach animals (Buckingham *et al.* 1978). Generally, the diets containing animal protein has growth promoting factor that enhanced the fish growth more than the diets containing plant protein irrespective to amono acids diffeciency in plant protein, where, the addition of methionine, lysine or cystine did not enhance the fish growth (Andrews and Page 1974).

The effect of incorporation level on carcass composition is presented in Table 3. The significant decrease in dry matter (DM%) and ether extract (EE%) strongly correlating with the increase of *Azolla* level in the diet, was in agreement with Micha *et al.* (1988) and El-Sayed (1992). Moreover, a significant reduction in protein content was also observed.

Table 3. Carcass proximate chemical analysis (mean ± SE) of *Tilapia zillii* fry fed on diets containing different levels of *Azolla* meal.

Diet	Item			
	DM (%)	CP (%)	EE (%)	ASH (%)
Initial	19.08 ± 0.24	52.09 ± 0.29	9.01 ± 0.07	36.74 ± 0.58
1	29.02 ± 0.10b*	62.01 ± 0.80a	13.31 ± 0.15a	24.41 ± 0.16e
2	23.99 ± 0.16a	59.84 ± 0.56b	11.95 ± 0.15b	26.91 ± 0.12d
3	20.11 ± 0.08c	59.88 ± 0.09b	11.22 ± 0.20c	28.86 ± 0.36c
4	19.62 ± 0.01c	58.41 ± 0.62b	9.86 ± 0.01d	30.47 ± 0.10b
5	18.43±0.48d	56.30±0.50c	9.68±0.13d	32.68±0.46a

* Figures in the same column not having the same letters are significantly different ($P<0.05$).

Feed intake was significantly affected by increasing *Azolla* level. This may reflect the selective appetite of *T.zillii*. *Azolla pinnata* var. *imbricata* ranked lower among other *Azolla* species fed to *T.rendalli* (Micha *et al.* 1988), and were nearly always neglected by *O.niloticus* (Antoine *et al.* 1986). The significant ($P<0.05$) decrease in PER, PPV% and EU% was a direct result of the increase of *Azolla* level.

This finding was in agreement with Micha *et al.* (1988) and El Sayed (1992).

Although fish gain in weight and specific growth rate (SGR%) were statistically equal at 0.0 and 25% *Azolla* level, the higher FCR and significantly lower values of PER, PPV and EU% correlating with 25% replacement of fish feed with *Azolla*, suggest a low nutritive value of *Azolla pinnata* for feeding of *T.zillii* fry, and confirm the conclusion of Buckingham *et al.* (1978) that, *Azolla* is not suitable as a protein source for simple-stomach animals. However, EL-Sayed (1992) suggested that, less than 25% of the dietary fishmeal protein may be replaced with *Azolla*, and recommended that, incorporation of *Azolla* in fish diet should be considered on cost/benefit ratio. Based on the results of the present study, an optimum inclusion level of *Azolla* meal should be incorporated at a level not more than 25% for the feeding of *T.zillii*. Moreover, taking into account the differences among fish species and age groups in their ability to digest plant materials (Caulton 1978, Buddington 1979), addition of Azolla to fish culture ponds should not absolutely be rejected, and more studies are needed for the best use of this costless protein source.

Table 4. Feed and nutrient utilized by *Tilapia zillii* fry fed on diets containing different levels of *Azolla* meal.

Diet	Feed intake (g/fish)	FCR	PER	PPV	EU %
1	10.38 ± 0.3	4.31±0.25d*	1.32 ± 0.08a	33.98±1.7a	15.70±0.8a
2	7.17 ± 0.4	4.21±0.22d	1.34 ± 0.06a	24.83± 1.1b	12.24±0.5b
3	4.29 ± 0.4	5.24±0.04c	1.07± 0.00b	18.93± 1.1c	9.74±0.5bc
4	2.28 ± 0.2	6.16±0.05b	0.91± 0.00bc	20.07±0.7bc	9.89±0.5bc
5	1.57 ± 0.16	7.12±0.41a	0.82 ± 0.05c	12.46± 1.2d	6.50±1.8c

* Figures in the same column not having the same letters are significantly different ($P<0.05$).

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تقييم مسحوق الازولا بياتا كمكوّن فى علائق البلطى الذيلى

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ربيت مجموعتان من البلطى الزيللى فى عشرة احواض زجاجية وغذيت على واحده من خمس علائق متساوية فى محتواها من النيتروجين لمدة ٩٠ يوما. احتوت العليقة القياسية على ٢٠٪ بروتين وقد تم استبدال جزء من العليقه بمسحوق الازولا بمعدل ٢٥,٠ ، ٧٥,٥٠ ، ١٠٠٠ ٪.

أظهرت النتائج تأخراً فى نمو الاسماك يزداد مع تزايد مستوى الازولا وكان معدل المنفوق ٢٣٪ للاسماك التى تغذت على ١٠٠٪ من مسحوق الازولا. أظهر التحليل الكيماوى لجسم الاسماك زياده فى نسبة الرطوبه والرماد ومحتوى اقل من البروتين والدهن. وكانت نسبة التحويل الغذائى عاليه ونسبة كفاءة البروتين والقيمه الانتاجيه للبروتين اقل مع زيادة مستوى الازولا. ويمكن استنتاج ان مسحوق الازولا ليس غذاءاً جيداً لزريعة البلطى الزيللى، ومع هذا يمكن استخدامه بمعدل اقل من ٢٥٪ كاحد مكوّنات علائق الاسماك على اعتبار أنه مصدر رخيص للبروتين.