



Growth and survival of pearlspot, *Etroplus suratensis* (Bloch, 1790) fry using practical diets incorporated with different levels of mosquito fern

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Received: 30 November 2022; Accepted: 18 January 2024

ABSTRACT

The present study was carried out to evaluate the growth and survival of pearlspot, *Etroplus suratensis* (Bloch, 1790) fry using practical diets incorporated with different levels of mosquito fern, for 60 days. The fry with average length and weight of 25 ± 0.1 mm and 0.54 ± 0.03 g, respectively were reared at stocking density 15 fry/100 L in brackishwater. Four iso-nitrogenous (30% crude protein) and iso-calorific diets were formulated with incorporation of 0, 10, 20 and 30% powdered fermented azolla. Fry were fed at the rate of 7-8% body weight per day. The highest growth parameters and survival were recorded for control diet. However, the fry fed 10% azolla incorporated diet showed similarities in weight gain %, specific growth rate and feed conversion ratio with control group.

Keywords: *Etroplus suratensis*, Fermented *Azolla pinnata*, Growth, Nutrition, Survival

Aquaculture is one of the fastest growing animal food production sectors and provides a high-quality protein source. Sustainable growth in this sector primarily depends on the culture of diversified finfish and shellfish species. Although many fish species are cultured in India, brackishwater species such as Asian seabass, pearlspot, milkfish, grey mullet, scat, estuarine grouper, cobia dominate Indian finfish aquaculture production. Some of the fish species have more demand in markets of specific region, e.g. Pearls spot which is important as a food fish (Costa 1983), has more demand in coastal parts of Kerala, Karnataka, Goa and Maharashtra (Rattan 1994). The fish can grow up to marketable size within a period of 7-8 months and fetches high market value up to ₹400-500/kg in Kerala (Patil *et al.* 2020). The important factors that contribute to higher cost of seed are the cost of fry and feed used during nursery phase. Low cost on-farm feeds can also be used in order to minimise expenses on feeding and cost of production of juveniles. The cost of farm-made feeds can be reduced by including low cost and easily available feed ingredients like mosquito fern. A mosquito fern, *Azolla* spp. is one of the naturally available plant sources (Mosha 2018). *Azolla* is a free-floating freshwater fern found in the tropics and subtropics. It can be cultivated easily (Rashad 2021). It has the potential to be very productive, with doubling time ranging from 1 to 5 days (Pouil *et al.* 2020). *Azolla* spp. have a higher crude protein content (ranging

from 19 to 30%), minerals, chlorophyll, carotenoids, amino acids (rich in lysine) and vitamins than most of the green forage crops and aquatic macrophytes and have favourable essential amino acid (EAA) composition for animal nutrition (Joysowal *et al.* 2018).

Considering the availability, cost effectiveness and nutritive value of mosquito fern, an attempt has been made in the present investigation to incorporate azolla at different levels in the practical diet of *E. suratensis* fry to find out its impact on their growth and survival.

MATERIALS AND METHODS

The present study was conducted in the Wet Laboratory, College of Fisheries, Shirgaon, Ratnagiri, Maharashtra ($17^{\circ}01'23.4''N$, $73^{\circ}17'55.0''E$). The pearlspot, *Etroplus suratensis* fry were sourced from ICAR-Central Institute of Brackishwater Aquaculture (CIBA) hatchery unit located at Vasai (West), District-Palghar, Maharashtra. The fry were acclimatized for a week in 500 L bio-secured fiberglass reinforced plastic (FRP) tanks with a salinity of 10 g/L. Pearls spot having average length and weight of 25 ± 0.1 mm and 0.54 ± 0.03 g, respectively were stocked at rate of 15 fry/L in 100 L of brackishwater. The fry were fed twice a day at a rate of 8% of body weight/day from day 1 to day 44. Whereas, the feeding rate was reduced to 7% of body weight/day from day 45 to day 60 (Patil *et al.* 2020). The faecal matter and uneaten feed were siphoned out and nearly 10-20% of water was exchanged every day.

An experiment was conducted by following completely randomised design (CRD). Four dietary treatments, viz. control (Basal diet without incorporation of *A. pinnata*),

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Table 1. Ingredient composition and proximate composition of four experimental diets

Ingredient(g/100 g)	Practical diet			
	T ₀	T ₁	T ₂	T ₃
Powdered fermented <i>A. pinnata</i>	-	10.00	20.00	30.00
Maize flour	19.00	15.00	12.00	9.00
Wheat bran	28.00	25.00	23.00	17.00
Soybean meal	13.00	11.00	11.00	11.00
Mustard cake	15.00	23.00	18.00	17.00
Fish meal	17.00	11.00	11.00	11.00
Shrimp meal	6.00	3.00	3.00	3.00
*Mineral mix	1.50	1.50	1.50	1.50
Guar Gum	0.50	0.50	0.50	0.50
<i>Proximate composition of diets (% dry matter basis)</i>				
Treatments	T ₀	T ₁	T ₂	T ₃
Crude protein (%)	30.05	30.1	29.83	30.36
Lipid (%)	5.95	5.55	5.7	5.8
Ash (%)	8.75	8.73	9.96	11.03
**NFE (%)	55.25	55.62	54.51	52.81
**Gross energy (kcal/g)	2.62	2.58	2.63	2.71

Soya oil was added @ 20 ml/kg in all the diets. *Mineral mix Uni-nutrich product of Sanzyme Biologics (P) Ltd., India. Mineral mix composition: Copper - 1.2 g/kg, Manganese - 3.8 g/kg, Iron - 7.5 g/kg, Zinc - 11.5 g/kg, Iodine - 0.6 g/kg, Calcium - 27.25%, Phosphorus - 7.45%, Selenium 0.01 g/kg, Cobalt - 98 mg/kg, Magnesium - 40 g/kg, Potassium - 40 g/kg. **NFE = 100 - (% crude protein + % crude lipid + % crude ash) (Bauer *et al.* 2012). **GE: Calculated after NRC (1993) as 5.64, 9.44, and 4.11 kcal/g for protein, lipid, and NFE, respectively (Abdel-Tawwab 2008).

T₁ (Fermented *A. pinnata* powder incorporated at the rate of 10%), T₂ (Fermented *A. pinnata* powder incorporated at the rate of 20%) and T₃ (Fermented *A. pinnata* powder incorporated at the rate of 30%) were used with five replicates for each group. Adequate aeration was provided by diaphragm pump to an individual tank.

Freshly harvested and washed mosquito fern was fermented as per the protocol given by Hundare *et al.* (2018). The fermented *A. pinnata* was solar dried and ground. Powdered fermented azolla was added in treatment diets at different levels 10, 20 and 30% whereas, control diet was formulated devoid of azolla. Diets were prepared with combination of different ingredients as presented in Table 1. The control diet and experimental diets were formulated to prepare iso-nitrogenous (30% crude protein) and iso-caloric diets. Proximate analysis of the diets was done by the standard methods (AOAC 1990).

Growth of survived fishes were recorded at interval of every 15 days. The individual length was measured in mm using scale. All survived the fry were weighed in mg by using electronic. Water quality parameters such as temperature, pH, dissolved oxygen, total alkalinity, salinity and free carbon dioxide were estimated fortnightly as per standard methods given by APHA (2005).

Mean values of the growth parameters (length, length

gain, weight, weight gain and specific growth rate, condition factor, coefficient of variation), survival, feed conversion ratio and protein efficiency ratio were analysed by one-way analysis of variance (ANOVA) using statistical software (SPSS 16.0 version). Data on condition factor, coefficient of variation and survival were arc-sign transformed and subjected to ANOVA. The difference was considered significant when P<0.05, and subjected to Tukey Post-hoc method.

RESULTS AND DISCUSSION

The data on growth, feed conversion ratio, protein efficiency ratio, survival, coefficient of variation and condition factor is given in Table 2. In the present study, the length gain (%) was found to be significantly better in fish fed diet without incorporation of azolla as compared to diet incorporated with azolla at rate of 10, 20 and 30% (P<0.05). The growth in terms of per cent weight gain and specific growth rate was similar in control and 10% powdered fermented azolla group (P>0.05). Similar feed conversion ratio of *E. suratensis* fry in control 0% (devoid of azolla) and 10% azolla groups was recorded in current study (P>0.05). Feed conversion ratio was significantly higher in 20% and 30% azolla groups than control group (P<0.05). The protein efficiency ratio and Survival (%) was found to be better in control group (devoid of azolla) when compared with groups incorporated with 10, 20 and 30% fermented azolla. The coefficient of variation and condition factor did not differ significantly in all the dietary groups (P>0.05).

In case of *Oreochromis niloticus*, higher length gain (%) of fry was recorded with 4% inclusion level of azolla in diet as compared to 0, 2 and 6% azolla dietary groups (Hundare *et al.* 2018). The growth in terms of per cent weight gain and specific growth rate was similar in control and 10% powdered fermented azolla group (P>0.05). The growth in terms of weight gain (%) and specific growth rate was similar in control and 10% powdered fermented azolla group (P>0.05). However, these parameters were found to be significantly lower in 20% and 30% powdered fermented azolla group in comparison with control group (P<0.05). The reduction in growth parameters in higher percentage of incorporation of azolla groups may be attributed to reduced digestibility on account of presence of anti-nutritional factors (Mohammadi *et al.* 2018, Kamali-Sanzighi *et al.* 2019, Magouz *et al.* 2020, Ahmed *et al.* 2022), high dietary fibre content (Kamali-Sanzighi *et al.* 2019, Magouz *et al.* 2020), increased metabolic rate and energy expenditure (Mohammadi *et al.* 2018, Magouz *et al.* 2020, Ahmed *et al.* 2022), low content and imbalance in amino acid make-up of azolla protein (Buckingham *et al.* 1978, Almazan *et al.* 1986, Olvera-Novoa *et al.* 1990, Viola *et al.* 1994, Fasakin *et al.* 2001), reduced food utilisation (Joseph *et al.* 1994) and higher lignin content (Ayyappan 2000). On the contrary, higher growth was recorded in fish fed diet with incorporation of azolla in comparison to control group (devoid of azolla) in some of the studies carried out

Table 2. Data on growth, feed conversion ratio, protein efficiency ratio, survival, coefficient of variation and condition factor

Growth parameter	Different treatments of Azolla			
	T ₀ (0% Azolla)	T ₁ (10% Azolla)	T ₂ (20% Azolla)	T ₃ (30% Azolla)
Initial body length (cm)	2.53±0.01	2.54±0.01	2.54±0.01	2.52±0.02
% length gain	73.91±2.01 ^b	58.90±1.60 ^a	63.34±1.64 ^a	57.86±2.25 ^a
Initial body weight (g)	0.55±0.02	0.54±0.01	0.54±0.01	0.54±0.01
% weight gain	187.17±11.12 ^b	151.83±9.92 ^{ab}	138.30±11.15 ^a	125.10±7.12 ^a
Specific growth rate (% day ⁻¹)	0.76±0.03 ^b	0.67±0.03 ^{ab}	0.63±0.03 ^a	0.59±0.03 ^a
Feed conversion ratio	4.23±0.22 ^a	5.21±0.24 ^{ab}	5.96±0.50 ^{bc}	6.93±0.48 ^c
Protein efficiency ratio	3.42±0.16 ^b	2.70±0.15 ^a	2.47±0.16 ^a	2.23±0.12 ^a
Survival rate (%)	96.00±2.67 ^b	89.33±2.67 ^a	86.67±2.98 ^a	81.33±1.33 ^a
Coefficient of variation	7.61±0.21 ^a	6.18±1.18 ^a	6.87±1.22 ^a	6.07±1.06 ^a
Condition factor	1.85±0.05 ^a	2.06±0.10 ^a	1.81±0.07 ^a	1.90±0.04 ^a

on *O. niloticus* (Abou *et al.* 2008), *Tilapia zilli* (Abdel-Tawwab 2008), *C. mrigala* (Gangadhara *et al.* 2014), *L. rohita* (Panigharhi *et al.* 2014), *Tor grypus* (Gokcinar and Bekcan, 2015), *L. frimbriatus* (Gangadhar *et al.* 2015), *L. rohita* (Kumari *et al.* 2017), *O. niloticus* (Hundare *et al.* 2018), *C. carpio* var. *communis* (Ahmed *et al.* 2022). Similar feed conversion ratio of *E. suratensis* fry in control 0% (devoid of azolla) and 10% azolla groups was recorded in current study ($P>0.05$). This increment in FCR of 20 and 30% azolla groups may be due to significantly lower live weight gain in *E. suratensis* fry as compared to control group over a period of 60 days. The lower weight gain and resultant high feed conversion ratio in these groups may be due to the presence of some growth inhibitors like anti-nutritional factors (Mohammadi *et al.* 2018, Kamali-Sanzighi *et al.* 2019, Magouz *et al.* 2020, Ahmed *et al.* 2022) and fibre content (Kamali-Sanzighi *et al.* 2019, Magouz *et al.* 2020) at higher levels. The results of the present study are substantiated with better feed conversion ratio recorded in groups of lower dietary incorporation of azolla. In the study of *C. mrigala* with azolla incorporation up to 20% (Gangadhar *et al.* 2014), *L. frimbriatus* with 10% of azolla inclusion level (Gangadhar *et al.* 2015), *O. niloticus* with 4% fermented azolla incorporation level (Hundare *et al.* 2018), *C. carpio* var. *communis* with 6.25% azolla inclusion level (Ahmed *et al.* 2022) showed better feed conversion was recorded.

The protein efficiency ratio in the present study was found to be better in control group (devoid of azolla) when compared with groups incorporated with 10, 20 and 30% fermented azolla. Reduction in protein efficiency ratio in azolla-based diets may be due to presence of anti-nutritional factors (Mohammadi *et al.* 2018, Kamali-Sanzighi *et al.* 2019, Magouz *et al.* 2020, Ahmed *et al.* 2022), imbalance of amino acid (Almazan *et al.* 1986) and low content of essential amino acid (Buckingham *et al.* 1978, Olvera-Novoa *et al.* 1990, Viola *et al.* 1994, Fasakin *et al.* 2001). In contradiction to the results of present study, higher protein conversion was obtained for 4% and 6.52% as of azolla inclusion groups in comparison to control in *O. niloticus* (Hundare *et al.* 2018) and *C. carpio* var. *communis* (Ahmed *et al.* 2022). However, protein conversion ratio was found

to be reduced at inclusion level more than 4% and 6.52%, respectively in these studies.

Survival of *E. suratensis* fry was found to be significantly higher in control group than azolla incorporated groups ($P<0.05$). However, mortality in the present study was found in each dietary group. The mortality may be attributed to increased stress level because of handling during length and weight measurements. The differences in mortality among dietary groups may be due to variation in number of fishes of different groups undergone higher handling stress. On the contrary, higher survival was recorded in azolla dietary groups than control group in *O. niloticus* (Abou *et al.* 2008), *L. rohita* (Panigharhi *et al.* 2014), *C. mrigala* (Gangadhara *et al.* 2014), *L. frimbriatus* (Gangadhar *et al.* 2015), *T. grypus* (Gokcinar and Bekcan, 2015), *O. niloticus* (Hundare *et al.* 2018), *C. carpio* var. *communis* (Ahmed *et al.* 2022). Improved palatability and acceptability of feed with incorporation of azolla in diet has been related to improved survival at 50% azolla in *L. rohita* (Panigrahi *et al.* 2014).

The coefficient of variation for length and condition factor did not show any significant variation ($P>0.05$) among all the groups. The finding of present investigation is also supported by similarity in condition factor of *T. grypus* fingerlings fed diet incorporated with azolla meal from 0 to 46% (Gokcinar and Bekcan 2015).

The optimum range of important water quality parameters such as salinity, pH, and temperature have been stated as 5 to 35 g/L, 7.5 to 8.5 and 28 to 32°C, respectively (Patil *et al.* 2020). The water quality parameter such as salinity, pH and temperature recorded in the current study were in the optimum range. Dissolved oxygen was recorded >3.2 mg/L suggested by Thirunavukkarasu and sultana (1997).

This can be concluded that control diet (devoid of mosquito fern, *Azolla pinnata*) showed better growth and survival in comparison to diets incorporated with 10, 20 and 30% of powdered fermented *A. pinnata*. A further experiment may be undertaken by considering incorporation level of mosquito fern between 0 and 10% in practical diet to find out its optimal level without affecting growth and survival of *E. suratensis* fry.

ACKNOWLEDGEMENTS

The authors thank Dr. Balasaheb Sawant Konkan Agricultural University, Dapoli, Ratnagiri and College of Fisheries, Shirgaon, Ratnagiri, Maharashtra, India for providing necessary facilities along with funds for conducting the study.

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