International Journal of Bio-resource and Stress Management 2018, 9(6):702-706

Research Article

Doi: HTTPS://DOI.ORG/10.23910/IJBSM/2018.9.6.1925

Natural Resource Management

Use of Fermented Azolla in Diet of Tilapia Fry (*Oreochromis niloticus*)

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Article History

Article ID: AR1925
Received in 29th October, 2018
Received in revised form 12th November, 2018
Accepted in final form 14th November, 2018

Abstract

The present study was undertaken to evaluate the effect of fermented azolla as fish meal substitute in the tilapia fry diet (*Oreochromis niloticus)* on growth performance, survival and feed utilization. In the experiment, the fry length initially ranging between (2.160±0.090 cm and 2.540±0.060 cm) and weight initially ranging between (0.190±0.010 g and 0.210±0.010 g) were fed on practical basal diet (30% crude protein) in the form of pellet during rearing period of 60 days. The fermented azolla were incorporated in the experimental diet at various levels such as 0% azolla, 10% fermented azolla, 20% fermented azolla and 30% fermented azolla to formulate four different test diets along with control diet. (Basal diet, without incorporation of fermented azolla, was used as control diet). The experimental fishes were fed with the experimental diet at of 7% of the body weight twice a day. Among the experimental treatments, the treatment 20% fermented azolla showed significantly higher growth parameters such as length gain (178.586±11.313%), weight gain (1346.770±70.251%), specific growth rate (4.449±0.083%), average daily growth (2.840±0.057%) and survival (95.556±2.222%). The feed utilization in terms of feed conversion ratio (1.236±0.030%) was significantly lower in 20% fermented azolla than control group, whereas protein efficiency ratio (4.145±0.169) in 20% fermented azolla showed significantly higher than that of other treatments.

Keywords: Tilapia, fermented azolla, growth, survival

1. Introduction

In broad terms, aquaculture production systems used for producing these aquatic animals and plants can be divided into feed-dependent systems or fed aquaculture (e.g. finfish and crustaceans) and non-fed aquaculture systems, where culture is predominately dependent on the natural environment for food, e.g. aquatic plants and molluscs (FAO, 2016). In Asia, fed aquaculture accounted for 54% of the region's total aquaculture production, indicating that almost half of Asia's aquaculture production comes from non-fed aquaculture. In contrast to the Asian situation, finfish and crustacean aquaculture production in Europe is 100% dependent on aquafeeds.

Asia is the largest global consumer of aquafeed ingredients (FAO, 2016). Aquaculture production, mainly of crustaceans and finfish, relies on farm-made or complete industrial diets. About two third of protein in aquatic diets comes from fish meal and its feeding value has caused its extensive use in aquaculture diets. Thus, with expansion of aquaculture industry, demand for fish feed has been increased with a huge pressure on fish meal industry. The reduction in catches of pelagic fishes, the sole resources of quality fish meal has resulted in rapid hike in prices which ultimately affected feed cost, thereby increase in operational cost of farming (FAO, 2016).

Fish nutritionists have made several attempts to partially or totally replace fish meal with less expensive, locally available protein sources. Due to the current high cost of fish meal, there is an intense pressure to re-evaluate common alternative protein sources to determine how best to use them in preparing low fish meal aquafeeds (Hardy and Tacon, 2002). There are several protein sources that have the potential of replacing fishmeal in aquaculture feeds without affecting the growth performance of fish (Tacon and Metian, 2009). In recent years, utilization of aquatic plants and weeds having high food values as feed ingredients has taken a new dimensions in producing the much required animal protein at low cost. The cost of production of aquafeed can be reduced using this ecofriendly and sustainable resources (Gangadhar et al., 2015).

Azolla has a higher crude protein content (ranging from 19 to 30%) than most green forage crops and aquatic macrophytes and rather favorable essential amino acid (EAA) composition for animal nutrition (rich in lysine), it has also attracted the attention of livestock, poultry and fish farmers (Cagauan and Pullin, 1991). Azolla seems to be rich in some vitamins, notably carotenes and vitamin A (Leonard, 1997 and Fiogbe et al., 2004).

Expansion of Tilapia culture is attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on wide range of foods. Even though, slight variations exist among tilapia species, nutrient requirements are primarily affected by the size of the fish (El-Sayed and Teshima, 1992). The global tilapia production in 2015 is 5.6 million tonnes and Asia contributes 4.1 mt in which Nile tilapia contributed 2.8 mt (FAO, 2017). The importance of Nile tilapia as an inexpensive protein source in rural communities, its geographical distribution, economic potential ability to reproduce in captivity, availability and in some countries inexpensive market price (Landau, 1992) has made Nile tilapia a preferred fish species for culture in many parts of the world (Fasakin et al., 2008).

A nutrient requirement for optimum growth for tilapia is dependent upon the quality of source, fish size or age and energy contents of the diets. The average protein requirement was varies from 45–50 (Stickney, 1997). The minimum requirement of dietary lipid in tilapia diets is 5% but improved growth and protein utilization efficiency has been reported for diets with 10%–15% (Ng and Chong, 2004). To improve the nutritional value of azolla for fish and subsequently to increase their incorporation level into fish diets, it is necessary to ferment the ingredient to improve rate of digestibility (Velasquez et al., 2011).

Research studies have shown that the protein of azolla plant is 23%–30% which includes 55% of the amino acid (Gokcinar and Bekcan, 2015). Considering the nutritional importance of azolla in fish diets, an attempt was made in the present study to replace fish meal partially by fermented azolla and to evaluate its effect on growth, survival, feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) of Tilapia.

2. Materials and Methods

The experiment was conducted in wet laboratory of College of Fisheries, Shirgoan, Ratnagiri. Fry of *Oreochromis niloticus* (Nile tilapia) ranging from 1.8±0.0098 cm to 2.0±0.0098 cm and 0.0552±0.0025 g to 0.91±0.0025g in length and weight respectively were obtained from Hans aquarium (Hasan Mhaslai, Gove village, Post- Pugaon, Tal- Roha, Dist- Raigad, Maharashtra, India). After transportation, acclimatization of fry was carried out to the lab conditions for a period of two weeks in 500 l capacity high density polyethylene (HDPE) circular tanks. Pelleted feed containing 30% CP was fed three times in day (07:00, 02.00 and 20:00 h) at the rate of 7% of body weight per day (Bag and Mahapatra, 2012). About 20 to 30% water exchange was carried out daily. Uneaten feed and faecal matter was removed by siphoning water before feeding. After acclimatization to laboratory conditions, initial length and weight of fishes were recorded before initiation of experiment.For the experiment, twenty one HDPE circular tanks having water holding capacity of 110 l were selected. Each tank was cleaned properly and filled up to the capacity of 100 L, for the experiment. The stocking density 30 fishes tank^{-1} was maintained during rearing period of 60 days.

A pelleted diet was prepared by using locally available feed ingredients. The proximate analysis of each ingredient was carried out (Table 1) to decide its inclusion rate in the basal diet containing 30% crude protein. After drying, grinding of each ingredient was carried out using electric grinder. The powder was sieved through 80 mesh to obtain fine powder required for feed formulation. The inclusion levels of ingredients in diet are shown in Table 2. The different ingredients were weighed separately and mixed thoroughly. Dough of mixed ingredients was prepared by addition of water at the rate of 100 ml 100 g^{-1} of feed mixture which was steam cooked for 15 minutes and cooled at room temperature (30 °C). The cooled dough was pressed through pellet machine to prepare pellets of 1.0 mm diameter. The pellets were spread uniformly on polythene sheet and kept at room temperature for cooling (Murkar, 2011). After 24 hours, pellets were separated out from the polythene sheet and oven-dried at 65 °C for 2 hours. After cooling, the pellets were packed in plastic pouches and stored in dry place.There are four iso-nitrogenous (CP 30%) practical diets were prepared (Table 3). Basal diet containing fish meal was treated as control while in remaining treatments, fish meal was substituted by 10% fermented azolla (T_1), 20% fermented azolla (T_2) and 30% fermented azolla (T_3).

Table 1: Proximate composition of ingredients used in experimental diets

Sl. No.	Ingredients	Protein	Lipid	Fibre	Moisture	Ash	NFE
1.	Fish meal	52.89	5.78	3.11	9.8	21.90	6.49
2.	Soybean meal	42.00	3.50	6.50	8.70	6.00	33.3
3.	Rice bran	21.65	1.82	8.43	9.6	12.08	46.42
4.	Azolla	28.59	3.47	15.71	10.1	15.76	26.37
5.	Groundnut oil cake	48.01	9.46	5.96	10.4	10.03	16.14
6.	Maize flour	16.27	2.76	1.36	9.0	4.75	65.86

Freshly harvested azolla (*Azolla pinnata*) was washed

Table 2: % composition of experimental diets

Sl. No.	Ingredients	Control diet	Fermented azolla feed		
		T_0	T_1	T_2	T_3
1.	Fish meal (59% CP)a	20	18	16	14
2.	Soybean meal (42% CP)a	15	16	17	18
3.	Groundnut oil cake	15	15	15	15
4.	Azolla	0	2	4	6
5.	Rice bran	24	22	20	18
6.	Maize flour	25	26	27	28
7	Salt	1	1	1	1

Table 3: Proximate composition (Dry weight basis) of experimental diets

Proximate Composition (%) on dry weight basis	T_0	T_1	T_2	T_3
Crude protein	30.81	30.81	30.25	30.6
Crude lipid	5.42	5.42	5.63	5.45
Crude ash	6.5	6.58	6.8	7.1
Crude fiber	15.16	15.34	16.34	15.94
NFE	42.11	41.85	40.98	40.91
Gross energy (MJ g^{-1})	1693.21	1688.64	1668.29	1668.27

thoroughly with tap water and kept in a airtight container. To initiate fermentation process, 5% yeast was added and mixed thoroughly. Fermentation process was carried out for four days in dark as per the protocol suggested byUtomo and Ekasari (2011). A fine paste was made after complete fermentation for its inclusion in diet, as feed ingredient.

2.1. Water quality parameters

Analyses of water quality parameters such as dissolved oxygen (DO), total alkalinity and free carbon dioxide (free CO_2) were estimated weekly using methods given by APHA (2005) and Boyd (1981). The water temperature and pH were recorded daily by using thermometer and Universal indicator respectively.

2.2. Statistical analysis

Data was expressed as the mean±SE of three replicates. Data recorded during the Experiment for growth parameters was analyzed by One-way ANOVA. Significant difference was indicated as $p<0.05$, among the treatments means (Snedecor and Cochran, 1967; Zar, 2006). The means were compared by using one way ANNOVA. The post hoc analysis was carried out by using Student's Newman Keul (SNK) of difference are significant $p<0.05$.

3. Results and Discussion

The growth performance, survival, feed utilization and water parameters of tilapia fry fed on diet supplemented with fermented azolla is presented in Table 4 and Table 5 respectively. The results showed that the diet containing 20% fermented azolla has shown showed highest growth, survival and feed utilization.

Table 4: Growth performance, survival and feed utilization

Sl. No.	Parameters	T_0	T_1	T_2	T_3
1	Length gain	96.516±8.130	111.242±8.774	178.586±11.313	153.285±20.041
2	Weight gain	927.346±43.982	1055.130±118.616	1346.770±70.251	1213.740±49.069
3	SGR	3.879±0.073	4.059±0.181	4.449±0.083	4.290±0.062
4	ADG	2.143±0.088	2.327±0.107	2.840±0.057	2.588±0.059
5	Survival	80.000±1.925	83.333±1.925	95.556±2.222	90.000±1.925
6.	FCR	1.814±0.067	1.556±0.048	1.236±0.30	1.359±0.042
7	PER	3.119±0.108	3.517±0.395	4.145±0.0169	3.935±0.199

Table 5: Water parameters observations during study period

Water parameters	Ranges	Mean values (±SE)
Temperature (°C)	26–28	26.07±0.19
pH	6–7	6.53±0.05
Dissolved oxygen (mg l^{-1})	4–6	5.83±0.12
Total alkalinity (mg l^{-1} as $CaCO_3$)	35–47	43.23±0.06
Free carbon dioxide (mg l^{-1})	1–3	1.52±0.08

The present research work is focused on protein nutrition of farmed tilapia with emphasis on the use of non-conventional, under-utilized protein sources. Azolla, an aquatic floating fern, which is widely distributed throughout tropical and subtropical zones. It rapidly propagates and may doubles its biomass in 3–10 days reaching a standing crop of 8–10 t ha^{-1} (Chen and Huang, 1987). This huge mass of high protein content has encouraged testing its use as fish fodder. Feeding tilapia with fresh or dried azolla has received special interest (Antoine et al., 1986; Pantastico et al., 1986; Antoine et al., 1987; Micha et al., 1988; Santiago et al., 1988 and El-Sayed and Teshima 1992). However, results were controversial. Various research studies have been conducted to evaluate the nutritional quality of azolla either in fresh or dry form. In majority of studies, azolla was used as a partial substitute for the costly protein rich sources. As per the results of the research investigations conducted by Antoine et al. (1986); Pantastico et al. (1986); Antoine et al. (1987); Micha et al. (1988); Santiago et al. (1988) and El-Sayedand Teshima (1992) have suggested that azolla can be effectively used as a feed ingredient. It can partially substitute the protein rich sources up to 30%. In order to increase the digestibility of feed in totality and feed ingredient individually, fermentation with the help of yeast was carried out in the present study. Utomo and Ekasari (2011) have pointed out that the process of fermentation improves the nutritional qualities with reduction in crude fibre level and increase in protein content. Therefore, in the present study, preliminary trial experiment was conducted to study proper duration required for fermentation viz. 4, 6, 8 and 10 days. Comparing the protein content, it was found that 4 days fermentation with yeast in air tight container was sufficient with a peak in protein content. In the present study, the decreasing protein content was observed 5^{th} day onwards. The results of trial experiments are fully in agreement with Utomo and Ekasari (2011).

In the different feeding trials conducted by Shiomi and Kitoh (2001), Fiogbe et al. (2004) and Abou et al. (2008) for fry of tilapia, azolla meal incorporation levels between 15 to 20% were proved to be suitable in terms of growth performance. After statistical analysis of the present experimental data, it was also proved that about 20% (fermented azolla paste) can be effectively used as a partial substitute for fish meal.

In other species such as *Tilapia zillii*, the incorporation level up to 25% was suggested by Tawwab (2008). Azolla can be used as feed ingredient and can be used as a protein replacer in fish species viz. *Labeo fimbriatus* (40%), *Tor grypus* (10%) and *Anabas testudineus* (15%) as stated by Gangadhar et al. (2015), Gokcinar and Bekcan (2015) and Mishra (2013) respectively.

Thus, based on the results of the present investigation and review of the earlier research works, it can be stated that fermented azolla can be efficiently substituted to fish meal up to 20% in dry form. In case of fermented azolla, the process of fermentation may increase the digestibility of nutrient subsequently feed ingredients and feed. This is evident from the results of the present study while comparing T_1, T_2, T_3 with T_4. Therefore it is proposed to use fermented azolla paste as a feed ingredient to increase the digestibility of feed in terms of lower feed conversion ratio and higher protein efficiency ratio.

4. Conclusion

Nile tilapia (*Oreochromis niloticus*) fed with four isonitrogenous diet containing 30% crude protein in which fish meal was partially substituted 10% fermented azolla, 20% fermented azolla and 30% fermented azolla in that20% fermented azolla showed better growth (length gain, weight gain and specific growth rate), survival and feed utilization such as feed conversion ratio (FCR) and protein efficiency ratio (PER) than other treatment during rearing for 60 days in laboratory condition.

5. Acknowledgement

The authors are thankful to the Collage of Fisheries Science, Shirgaon, Ratnagiri, DRBSKKV, Dapoli, for their support, suggestions and encouragement during the research.

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