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Effect of total replacement of fishmeal by earthworm and *Azolla filiculoides* meals in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) reared in concrete tanks

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ABSTRACT

In order to eliminate use of fishmeal in aquafeeds, we evaluated the effect of total replacement of fish meal by a mixture of earthworm (*Eisenia foetida*) and aquatic fern *Azolla filiculoides* in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758). The experiment was conducted in *O. niloticus* fingerlings (initial mean weight = 5.6±0.2 g) for a period of 60 days. Five experimental diets isoproteic (30.4±0.6% protein) comprising control diet A0 and four test diets (A1 to A4) without fish meal were formulated. To satisfy essential amino acids requirements in test diets, the ratios 2:1; 1:1; 1:2; 1:5 between *A. filiculoides* and earthworm meals were used respectively in total replacement of fish meal. The diets were tested in triplicate. On termination of the experiment, the final mean weights in the test diets (10.79 to 11.94 g) were lower than 14.9 g obtained in group fed control diet ($p<0.05$). The best growth performance and feed utilisation were obtained in fish fed test diets A1 and A4 (SGR = 1.25 and 1.26% day^{-1}; FCR = 2.37 and 2.31 respectively). Excess of lysine in test diets was found to affect the feed utilisation.

Keywords: Feed utilisation, Growth performance, Iisoproteic, Total replacement

Introduction

Aquaculture is regarded as one of the solutions to alleviate overexploitation of natural fishery resources, but is confronted with many issues. The cost of feed represents a significant part of the production cost, which limits the economic value of aquaculture (Tacon,1996; Hoffman *et al.*, 1997; Siddhuraju and Becker, 2003; Fiogbe *et al.*, 2004). Fishmeal is the major ingredient that contributes to the cost of feeds (Bamba *et al.*, 2008), which is used as the main source of protein in fish feeds due to its richness in essential amino acids (EAAs) which meets the nutritional requirements of fish (El-Saidy and Gaber, 2004; Azaza *et al.*, 2005; Imorou Toko *et al.*, 2008; Abarike *et al.*, 2013). The use of agricultural byproducts to replace fishmeal has already yielded encouraging results (Medale *et al.*, 2013). However, the feed formulae developed based on these products often meet the protein requirements of fish regardless of the satisfaction of EAA requirements, which are essential for obtaining good growth performance in fish.

Azolla filiculoides is an aquatic fern, rich in proteins (Alalade and Iyayi, 2006) with a good profile in amino acids (Accodji *et al.*, 2009; Leterme *et al.*, 2009) and increasingly being utilised in developing fish feed (Abou *et al.*, 2007; Abou *et al.*, 2010). Likewise, earthworms are animal protein sources with high nutritive values (Sogbesan *et al.*, 2007). Also they have high digestibility (NRC, 2011) and good EAA contents (Adesina, 2012). Nile tilapia (*Oreochromis niloticus*) is an omnivorous species with herbivorous tendency, well appreciated, with strong economic potential and good growth (Djissou *et al.*, 2016). The present work aimed to investigate the effect of total replacement of fish meal by earthworm (*Eisenia foetida*) and *Azolla filiculoides* meals in *O. niloticus* fingerlings.

Materials and methods

Experimental set up

This study was conducted at the research station at the University of Abomey-calavi. Fifteen circular concrete tanks, each having 500 l capacity were used to conduct the experiment. Each tank was filled with 300 l of water with a flow rate of 3 l min^{-1}. Surface of each basin was half covered with a rack in order to avoid direct solar light which helped to avoid large variations in water temperature as well as development of algae.

A total of 750 nos. of *O. niloticus* fingerlings with initial average weight of 5.6±0.2 g were acquired from the Research Innovations Centre for Agriculture, Breeding and Fishing Development of Takon (Oueme). The fish were acclimatised for a period of one week in the laboratory. The stocking density was 50 fingerlings per tank and each diet was tested in triplicate. Fish were manually fed with experimental diets at the rate of 5%, twice per day (08 00 and 17 00 hrs) for a period of 60 days.

The water quality parameters *viz*., pH, dissolved oxygen and temperature were monitored once in every three days. The mean values of these parameters during the experimental period were 5.62±0.8; 5.42±0.73 mg l^{-1} and 27.60±0.66°C respectively for the pH, dissolved oxygen and temperature in the rearing tanks. Once every ten days, the total number of survivors in each tank was counted and fish biomass determined.

Experimental diets

Cotton seed meal, corn bran, rice bran, soybean meal and starch produced locally were the basal ingredients used in the diets. The fishmeal used was obtained after milling fish (*Sardinella maderensis*) bought from Dantokpa market. Brewer yeast and chicken viscera waste were procured from private companies. *Azolla filiculoides* and chicken viscera meals were dried following the methods described by Hedji *et al*. (2014a; 2014b). Earthworms produced at the Research Station were washed, lyophilised before use. Four diets (A1, A2, A3 and A4) without fish meal and a control diet A0 containing fishmeal, all isoproteic were prepared to feed *O. niloticus* fingerlings. To satisfy EAA requirements in test diets, the ratios 2:1; 1:1; 1:2; 1:5 between *A. filiculoides* and earthworm meals were used respectively in total replacement of fish meal (Table 1).

Biochemical analyses

The bromatological analysis and amino acid composition of the feed ingredients were done as per AOAC (1990) and that of Fiogbe (2009) respectively. The proportion of EAA of the different experimental diets estimated are presented in Table 2. All of these amino acid analyses were conducted at the Laboratory of Aquatic Animal Nutrition, University of Kagoshima, Japan.

Feed processing

Raw ingredients were finely ground and sieved (355µm). For each diet, the ingredients were weighed, mixed to obtain a homogeneous powder and then palm oil was added and mixed further. Water was then added @ 50% of dry matter to obtain a malleable paste. This paste was then made into the shape of noodles (2 mm dia) by passing through a mincer (Moullinex HV8). The prepared

Table 1. Composition of the diets (g kg dry matter^{-1})

Ingredients	A0	A1	A2	A3	A4
Azolla filiculoides	0	200	150	100	50
Chicken viscera	0	50	30	10	0
Rice bran	150	22	42	62	72
Fish meal	300	0	0	0	0
Soybean meal	150	150	150	150	150
Brewer's yeast	0	100	100	100	100
Cotton seed meal	150	150	150	150	150
Maize bran	150	150	150	150	150
Earthworm meal	0	100	150	200	250
Palm oil	50	30	30	30	30
Vitamin/Mineral	0	10	10	10	10
Starch	50	20	20	20	20
Methionine	0	7	7	7	7
Lysine	0	11	11	11	11
Ratio*	-	2 :1	1:1	1:2	1:5
Biochemical composition					
Crude protein (%)	29.8	30.5	30.8	31.0	30.9
Crude lipid (%)	10.76	10.10	10.05	10.00	10.13
Energy (kj g^{-1})	12.98	12.67	12.36	12.29	12.71

*: Ratio between *Azolla filiculoides* and earthworm meals

Table 2. Essential amino acids (EAA) composition of the experimental diets (% of dry diet)

EAA	A0	A1	A2	A3	A4	*O. niloticus* requirement*
Threonine	0.83	0.87	0.88	0.90	0.91	1.05
Valine	0.94	0.85	0.83	0.82	0.82	0.78
Methionine	0.57	0.99	1.00	1.01	1.03	0.75
Isoleucine	1.33	0.98	0.98	0.99	0.91	0.87
Leucine	1.52	1.58	1.60	1.62	1.65	0.95
Phenylalanine	1.47	1.12	1.12	1.13	1.11	1.05
Histidine	0.75	0.70	0.71	0.71	0.71	0.48
Tryptophane	0.32	0.34	0.31	0.28	0.24	0.28
Lysine	1.40	2.53	2.41	2.46	2.47	1.43
Arginine	1.79	1.69	1.70	1.71	1.68	1.18

*NRC (1993; 2011)

feeds were then sun-dried, fragmented to desired size, packed in sealed containers and stored at 4°C until used for the experiment.

Estimation of growth, survival and feed utilisation

Growth performance, survival and feed utilisation in the different diet groups were calculated as:

Specific growth rate (SGR% day^{-1}) = [(ln FBW - ln IBW)/ T] × 100

where, FBW and IBW = final mean weight and the initial mean weight, respectively; T = feeding period in days.

Survival rate (SR %) = [(Nf / Ni) × 100]

where, Nf = final total number of fish;
Ni = initial total number of fish.

Feed conversion ratio (FCR) = Total feed intake (g) / Total weight gain (g)

Protein efficiency ratio (PER) = Total weight gain (g) / Protein intake

Statistical analyses

Statistical software Statview (version 5.01) was used with the threshold of probability of 5%. The variance analysis to a criterion of classification (ANOVA 1) was used to compare the zootechnic performances of the various diets. Test LSD of Fisher was used to carry out pair comparisons of the various means.

Results

The results in terms of growth performance and feed utilisation obtained are summarised in Table 3. The final average weight varied significantly with the treatment. Final average weights obtained with diets containing earthworm and *A. filiculoides* meals (A1 to A4) were found to be significantly lower ($p<0.05$) compared to that obtained with the control diet A0 containing fish meal (Fig. 1).

Table 3. Growth performance and feed utilisation of *O. niloticus* fed with the experimental diets

Parameters	A0	A1	A2	A3	A4
IBW (g)	5.65±0.3^{a}	5.71±0.14^{a}	5.55 ±0.10^{a}	5.70 ±0.17^{a}	5.73±0.14^{a}
FBW (g)	14.9±0.48^{a}	11.88±0.46^{b}	10.79±0.54^{b}	11.09±0.18^{b}	11.94±0.06^{b}
SGR (% j^{-1})	1.64± .10^{a}	1.26±0.10^{b}	1.11±0.10^{b}	1.14±0.03^{b}	1.25±0.04^{b}
FCR	1.83±0.09^{a}	2.37±0.09^{bc}	2.79±0.28^{b}	2.39±0.05^{bc}	2.31±0.04^{c}
PER	1.84±0.01^{a}	1.35±0.01^{b}	1.19±0.02^{c}	1.25±0.02^{c}	1.39±0.01^{b}
SR (%)	86±1.15^{a}	95.33±2.66^{b}	90.67±2.90^{ab}	94±1.15^{b}	91.33±2.91^{ab}

Values are expressed as Mean±SD
Values bearing same superscripts in the same row are not significantly different ($p>0.05$)

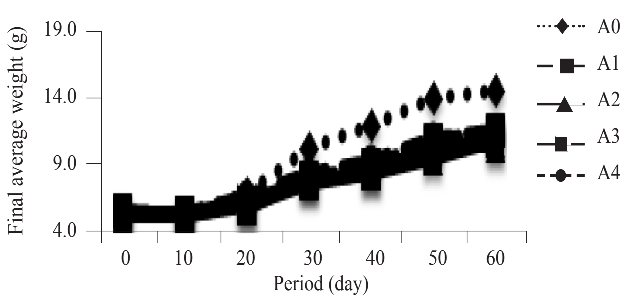

Fig. 1. Growth of *O. niloticus* fed with different experimental diets

The highest SGR was obtained with fish fed with the control diet ($p<0.05$). No significant difference was observed between the SGR obtained with the diets A1, A2, A3 and A4 ($p>0.05$).

Discussion

Fish fed with all test groups accepted the test diets in which fishmeal was totally replaced by a mixture of earthworm meal and *A. filiculoides* meal. The results clearly showed that the test diets without fishmeal did not negatively affect fish growth. However, control diet containing fish meal led to better growth compared to diets A1 to A4 where fish meal was completely replaced by a mixture of earthworm and *A. filiculoides* meals. Differences in growth performances and feed utilisation of *O. niloticus* fingerlings fed with the control diet (A0) compared to those fed with test diets (A1 to A4) are explained by quality of protein sources used as well as digestibility of raw materials. Amino acid profile of fishmeal meets the needs of fishes (Burel and Medale, 2014) and contains EAA in sufficient quantities for the growth of fish (Green *et al*., 2002) in contrast to plants (such as *A. filiculoides*) which are notably deficient in lysine and methionine.

All the experimental diets met the EAA requirements of *O. niloticus* (NRC, 1993) except in threonine. Although diets A0 and A4 were also deficient in methionine and tryptophan (Met 0.57% and Trp 0.24% respectively), but the best growth performance and feed utilisation of *O. niloticus* fingerlings were obtained with these diets (SGR -1.64 and 1.25% day^{-1}; FCR -1.83 and 2.31; PER - 1.84 and 1.39 respectively). These results do not agree with the study of Ronyai *et al*. (2002) and Fagbenro (2004) who opined that the deficit in one or more amino acids affects protein synthesis and growth of fish.

In addition, the excess of lysine in diets A1 to A4 due to the earthworm and *A. filiculoides* meals could also explain the comparatively lower growth performances obtained in *O. niloticus* fingerlings fed with these diets compared to those fed with diet A0. In fact, the excess of lysine increases the activity of arginase and thus the need for arginine, creating a lysine-arginine antagonist effect in chicken and rats (Kestemont, 2007). When the need for arginine increases, the synthesis of polyamines involved in the regulation of growth reduces. Thus, the excess of lysine (2.47%) in diet A4 may explain the difference in growth observed with the control diet A0 whose lysine level (1.40%) is close to lysine requirement of *O. niloticus* fingerlings (NRC 1993; NRC, 2011).

Sitasit and Sitasit (1977) asserted that the best growth performance in fish is obtained when the ratio of vegetable proteins to animal proteins is low. This justifies the better performance obtained with *O. niloticus* fingerlings fed with diet A4 (ratio 1:5) compared to other diets A1, A2 and A3. The ratio 1:4 between vegetable protein and animal protein as a complete replacement for fish meal seems acceptable in *O. niloticus* (Stickney, 1986).

Results of the present study are in agreement with the results observed by Davis and Stickney (1978) in *O. aureus* and by Mathavan and Paudian (1976) in *O. mossambicus,* who reported that the growth performance is greatly improved following an increase in the incorporation rate of animal proteins. Mukti Pada *et al*. (2012) observed comparatively low SGR of 0.96% day^{-1} in *O. niloticus* fed diet incorporated with earthworm meal @ 32.2%. Total replacement of fish meal by earthworm meal in carnivorous fish led to SGR of 1.3 and 0.74 in juveniles of *Heteroclarias* (Monebi and Ugwemba, 2013) and *Parachanna obscura* fingerlings (Vodounnou *et al*., 2016) respectively.

Presence of fibers, anti-appetite substances and anti-nutritional factors (Pucher, 2014) in the raw materials used would hamper the digestibility of feed and thus the growth performance obtained. According to Bamba *et al*. (2008) and Koprucu and Ozdemir (2005), the digestibility of feed depend on the nature of raw materials used. These authors asserted that raw materials may appear to be an excellent source of nutrients but of low nutritional value due to variability in digestibility, absorption and availability of nutrients (amino acids, minerals). *A. filiculoides*, an aquatic fern, has low cellulose content but may contain high concentrations of lignin (Abou, 2007), which would affect growth performance. Indeed, the presence of these limiting factors reduce the acceptability and ingestion of feed, which interfere with complex forms of protein, making them less available to fish (Francis *et al*., 2001; Kashmir *et al*., 2003). Keembiyehetty and de Silva (1993) and Yousif *et al.* (1994) have shown that certain mechanisms determine the efficiency of digestibility and assimilation of plants because of their high fiber content (cellulose). The presence of anti-nutritional factors in materials of vegetable origin directly (trypsin inhibitors, phytic acids) or indirectly (fibers, tannins, glucosinolates) affects the digestive capacity of fish (Francis *et al.,* 2001). The diet efficacy depends not only on its chemical composition and palatability, but also on its ability to be digested and absorbed through the intestinal mucosa.

Results of the study clearly showed that a mixture of animal and plant proteins in the feed of *O. niloticus* fingerlings by total replacement of fish meal can meet the EAA requirements and can also lead to acceptable animal performance. The study also indicated the potential of replacement of fishmeal (which is nowadays expensive and of poor quality) in aquaculture feeds by non-conventional ingredients. Nevertheless efforts need to be continued to satisfy all EAA requirments and to improve the digestibility of ingredients of plant origin in obtaining better growth performances and feed utilisation in fish.

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