Aquaculture Reports 5 (2017) 18–26

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Evaluation of *Imbrasia belina* meal as a fishmeal substitute in *Oreochromis mossambicus* diets: Growth performance, histological analysis and enzyme activity

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ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form 4 November 2016
Accepted 20 November 2016
Available online 4 December 2016

Keywords:
Insects
Digestibility
Mopane worm
Fish
Cost

ABSTRACT

The main objective of this study was to investigate mopane worm (*Imbrasia belina*) as a protein source in the diet of *Oreochromis mossambicus*. One thousand five hundred *O. mossambicus* fingerlings (40 ± 2.5 g) were fed five isonitrogenous, isolipidic and isoenergetic diets formulated to contain 30% crude protein and 20 MJ/kggross energy (dry matter basis) for 51 days. Fifteen indoor rectangular concrete tanks (1.5 m^3) connected to a recirculating system were used. Water temperature ranged between 27 and 29 °C. The diets were prepared by replacing fishmeal with mopane worm meal at 10%, 20%, 40% and 60%. The diets were coded D2, D3, D4 and D5 respectively. A control diet with no mopane worm meal was coded D1. The diets were fed to triplicate groups of *O. mossambicus* twice a day. Specific growth rate (SGR), Thermal-unit growth coefficient (TGC), protein efficiency ratio (PER) and apparent digestibility coefficient (ADC) increased with higher inclusion levels of mopane worm meal. Feed conversion ratio (FCR) also improved with higher inclusion levels. However, the highest growth performance (SGR: 3.49%; FCR: 1.29) was recorded in fish fed the fishmeal based control diet. Protease, amylase and lipase activities were determined in the intestines. Both protease and amylase activity were significantly higher ($P < 0.05$) at high mopane worm inclusion levels. It is suggested that the high protein levels of the mopane worm diet elicited high protease activity. The health status of the fish was evaluated by examining the liver and intestine histology. There were no evident histological alterations of either liver or intestine as mopane worm meal inclusion levels increased. This showed that mopane worm meal may be a good candidate for the replacement of fishmeal in *O. mossambicus* diets. The highest profit index (1.67) was recorded in the 60% mopane worm inclusion level. The lowest profit index was in the control. More studies on mopane worm meal as a substitute of fishmeal are recommended in other fish species.

1. Introduction

Fish feed generally accounts for 40–70% of the total production costs in aquaculture (Rana et al., 2009). Fishmeal is the most expensive component of fish feed. The unprecedented increase in the price of fishmeal triggered by the decline of fish stocks in the wild has made the search for locally available alternatives an international research priority. Plant based protein sources are much cheaper than animal based protein sources, however, they are deficient in essential amino acids especially lysine and methionine. Anti-nutritional factors such as tannins, sapponins and fiber reduce feed digestibility and this leads to lower fish growth (El-Sayed, 1999; Naylor et al., 2009: Merrifield et al., 2009). For this reason, animal protein sources that can replace fishmeal are increasingly getting attention.

The criteria used to select potential novel ingredients to replace fishmeal are high protein and lipid content, balanced amino acid profile and high digestibility of the novel ingredient. Based on these criteria the potential of insect protein as partial or complete replacement for fishmeal was recently highlighted (Sanchez-Muros et al., 2014; Riddick, 2014; Henry et al., 2015). Black soldier fly (St-Hilaire et al., 2007a; Sealey et al., 2011; Kroeckel et al., 2012), housefly maggot (Fasakin et al., 2003; Sogbesan et al., 2006), silkworm pupae (Nandeesha et al., 2000; Zhang et al., 2008), superworm (Jabir et al., 2012) and yellow mealworm (Ng et al., 2001) are some of the insect proteins that have been used for replacement of fishmeal in fish diets. It has been reported that percentages of fishmeal replacement of over 25% reduced the growth of fish (St-Hilaire et al., 2007b; Alegbeleye et al., 2012). The magnitude of the replacement level depends on the insect species and fish species.

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http://dx.doi.org/10.1016/j.aqrep.2016.11.004

The mopane worms, that have been reported to have high protein and lipid content (Moreki et al., 2012), may be an interesting candidate for inclusion in fish diet. In Southern Africa the mopane worms (*Imbrasia belina*) are widely distributed in Botswana, Namibia, South Africa, Zimbabwe, Mozambique and Zambia. They are the larvae of emperor moths. Mopane worms feed on the leaves of mopane trees (*Colophospermum mopane*) (Gondo et al., 2010). However, there are hardly any studies that have been undertaken on mopane worms as fishmeal substitute in fish diets. This is despite mopane worm's high protein content, good amino acid profile and high lipid content. Availability of mopane worms is seasonal. Mopane worm outbreaks occur between November to May. In order to ensure all year round availability of mopane worm, mass rearing of mopane worms is currently being investigated in South Africa, Botswana and Zimbabwe.

The test fish species in this study was *Oreochromis mossambicus*. It is one of the most widely cultured tilapia species (FAO, 2013). *Oreochromis mossambicus* can feed on live aquatic and submerged terrestrial plants, benthic algae, phytoplankton, zooplankton, insects and organic detritus (Kim et al., 2002; Bowen, 1981; De Silva et al., 1984). The fish undergoes a size related ontogenic shift and predominantly feeds on phytoplankton in the adult stage (Trewavas, 1983). Recently its ability to use aquatic insects was demonstrated in organically manured aquadams (Rapatsa and Moyo, 2015). No studies have been undertaken on the use of mopane worm as a protein source in the diet of *O. mossambicus*. In this study, the growth performance of *O. mossambicus* to graded levels of mopane worm meal was determined. Furthermore, the effect of mopane worm meal on the liver and intestine histology was determined. The capabilities of *O. mossambicus* to digest the mopane worm meal were investigated by determining proteinase, amylase, lipase and chitinase activities. Cost/benefit analysis of using mopane worm meal as a fishmeal substitute was also undertaken.

2. Materials and methods

2.1. Diet preparation

The mopane worms were purchased at a market in Thohoyandou, Vhembe district, Limpopo Province, South Africa. Traditionally, mopane worms are harvested from the wild, degutted, cooked in brine and then sun dried. The mopane worms bought at the market had been processed in this way. The worms were analyzed for proximate composition following procedures stipulated by AOAC International (2003). Five diets were formulated by replacing fish meal with mopane worm meal at 10, 20, 40 and 60%. The diets were formulated to be isonitrogenous, isocaloric and isolipidic. The control diet comprised of 40% fish meal with no mopane worm. Fishmeal was put at 40% to achieve significant replacement responses. Ingredients were weighed, triturated and mixed; mixtures were then pelleted using a meat grinder to form pellets. The pellets were sun dried and stored in plastic containers at − 10 °C until use. Diets were denoted D1 (0%), D2 (10%), D3 (20%), D4 (40%) and D5 (60%). Maize, wheat bran and maize gluten levels were adjusted accordingly (Table 1). Chromic oxide (Cr_2O_3) was added at 0.5% in each diet as an inert marker. Diets were formulated using Winfeed 3, EFG (Natal).

2.2. Experimental design

The experiment was carried out at the Aquaculture Research Unit (ARU), University of Limpopo, South Africa for 51 days. All male *Oreochromis mossambicus* were obtained from the ARU hatchery and were fed a commercial diet (CP 34.48%; Gross energy 17 MJ/kg) until they reached the desired juvenile size. Fifteen indoor rectangular concrete tanks (1.5 m^3) connected to a recirculating system were used. The recirculating system consisted of 78 rectangular self-cleaning concrete tanks. Aged municipal water was pumped to each tank at a rate of 1L per minute from two bio-filters each with a capacity of 339.29 l. Each tank was filled with aged water up to the 1 m^3 mark. The experiment was set up in a completely randomized design. Each diet was randomly assigned to triplicate groups of *O. mossambicus* (40 ± 2.5 g) stocked at 100 fish per tank. The fish were fed their allocated diets twice a day (09:00 h and 15:00 h) to apparent satiation (one pellet remains uneaten for 1–2 min) and the amount of feed consumed recorded for each tank. Faecal samples were siphoned from each tank 2 h after feeding and stored in a freezer (−20 °C) until enough faeces were collected for analysis. Water temperature ranged between 27 and 29 °C, dissolved oxygen was 6–9 mg/l, pH was 7–8 and the photoperiod was natural.

Table 1
Ingredients composition (g/kg) and calculated nutrients of the experimental diets.

	FM100:MOP0	FM90:MOP10	FM80:MOP20	FM60:MOP40	FM40:MOP60
Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	40.00	36.00	32.00	24.00	16.00
Mopane worm	0.00	4.00	8.00	16.00	24.00
Maize	31.90	32.29	32.69	33.48	33.59
Wheat bran	3.56	2.68	1.79	0.01	0.00
Maize gluten meal	0.04	0.53	1.02	2.01	3.52
Soybean meal	5.00	5.00	5.00	5.00	5.00
Rapeseed meal	5.00	5.00	5.00	5.00	5.00
Rapeseed expeller	5.00	5.00	5.00	5.00	5.00
Sunflower	5.00	5.00	5.00	5.00	3.39
Rapeseed oil	1.00	1.00	1.00	1.00	1.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00	2.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50
Crude protein (% DM)	30.00	30.00	30.00	30.00	30.00
Gross energy (MJ/kg)	20.00	20.00	20.00	20.00	20.00
Fat (% DM)	12.00	12.00	12.00	12.00	12.00
Ash (% DM)	8.60	8.50	8.00	7.70	7.10
Dry matter (%)	93.69	91.40	90.90	90.50	90.10

FM-fish meal; MOP- Mopane worm (*Imbrasia belina*)
Vitamin premix (g or IU kg-1 premix): thiamine, 5; riboflavin, 5;niacin, 25; folic acid, retinol palmate, 500,000 IU;1; pyridoxine, 5; cyanocobalamin, 5; cholecalciferol; 50,000 IU; a-tocopherol, 2.5; menadione, 2; inositol, 25; pantothenic acid, 10; ascorbic acid, 10; choline chloride, 100; biotin, 0.25.