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RESEARCH ARTICLE

Evaluation of Blowfly (*Chrysomya megacephala*) Maggot Meal as an Effective, Sustainable Replacement for Fishmeal in the Diet of Farmed Juvenile Red Tilapia (*Oreochromis* sp.)

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Fishmeal is the primary protein source used in farmed fish feed. However, the high price of fishmeal is a major contributor to high production costs in the aquaculture industry. Insect-based diets have been recognized as cheaper alternatives to fishmeal, but the nutritional quality and impact on fish growth remains in question. In this study, the potential of blowfly Chrysomya megacephala maggot meal as an alternative dietary protein source to fishmeal for red tilapia (Oreochromis sp.) was evaluated. The protein and amino acid composition of the meal was assessed and a feeding trial was conducted to determine the effects of varying percentages of fishmeal replacement on growth, feed efficiency, and survival of juvenile tilapia. Blowfly maggot meal contained all the essential amino acids needed by juvenile tilapia for normal growth, and equivalent protein content to fishmeal. Furthermore, diets with increased replacement of fishmeal by blowfly maggot meal improved the growth, feed efficiency and survival of juvenile tilapia with the total replacement diet giving the optimal results. Based on these results we suggest that blowfly maggot meal is an effective and sustainable protein source to replace fishmeal in the diet of farmed tilapia.

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INTRODUCTION

Tilapia (*Oreochromis* sp.) is a popular food fish and is in great demand globally. Global tilapia production reached 3.2 million tons in 2010 and the demand for tilapia is still growing, especially in the United States, the largest single market for tilapia (Watanabe *et al.*, 2002; FAO, 2011). The high demand has prompted countries such as China, Malaysia, Brazil, Thailand and the Philippines to invest in tilapia culture (Watanabe *et al.*, 2002) which in turn has elevated tilapia to second place amongst the farm-raised food fish in the world by volume (FAO, 2011). Selective breeding programs and genetic improvement technology have also been implemented to enhance the production of tilapia (Gupta and Acosta, 2004; Tawfik, 2013; Shaukat and Javed, 2013).

In fish feed, protein is an important dietary nutrient for growth and general good health (Marley, 1998; Khan *et al.*, 2012; Naz and Javed, 2013). Typically, fishmeal and soybean meal are the most commonly used protein sources in farmed fish feed. Fishmeal has high nutritional value as it contains at least 50% crude protein and all the essential amino acids required by fish (Marley, 1998). On the other hand, plant proteins contain low levels of sulfurcontaining amino acids (cysteine, methionine and taurine) which are needed by fish (Brinker and Reiter, 2010). Therefore, fishmeal is the primary protein source for the formulation of farmed fish feed (Li *et al.*, 2009). However, the high price of fishmeal is a major contributor to high production costs in the aquaculture industry (Hardy, 2010; Elnwishy *et al.*, 2012).

The global supply of fishmeal has dwindled due to overexploitation of the natural fishery stock. With the predicted continuous growth of the aquaculture industry (Brugère and Ridler, 2004), the demand for fishmeal will continue to increase, causing its price to soar. An estimated one-third of wild caught fish is used to produce fishmeal for aquafeed (Tacon, 1998). The poultry and swine industry is the largest fishmeal consumer (Hardy and Tacon, 2002) but the protein requirement for aquafeed is much higher than for livestock feed. The increasing demand for fishmeal (Tacon and Metian, 2008) has placed the pelagic fishes, which are the major source of fishmeal, in endangered status. The rapid growth of the aquaculture industry has changed fish capture patterns from large piscivorous fishes to smaller invertebrates and planktivorous fishes but this has not alleviated the pressure on wild fisheries stocks (Pauly *et al.*, 1998). This clearly presents a threat to marine ecosystems as well as a constraint to the long-term growth of the aquaculture industry itself.

It is, therefore, crucial that alternative protein sources be found to reduce feed costs, and to make aquaculture a viable, sustainable and attractive venture (Millamena, 2002; Richard *et al.*, 2011). An alternative protein source should be easy to obtain and be obtainable in sufficient quantities to meet demand. Additionally, the amino acids derived from the alternative protein source should meet the basic amino acid requirements of fish. The palatability of the protein source should be equivalent to fishmeal to avoid rejection by the fish.

Insect-based diets have been recognized as one of the cheaper alternatives to fishmeal. Insects such as the mealworm beetle (*Tenebrio molitor*) and the house fly (*Musca domestica*) have been studied as alternative protein sources in fish diets with promising results (Ng et al., 2001; Zuidhof et al., 2003; Ogunji et al., 2008). Furthermore, in the wild, insect larvae are the natural food sources for many animals including some fish (Srivastava et al., 2009). Blowfly (*Chrysomya megacephala*) maggots can be found on decomposing carcasses and use enzymes to convert the tissues into a form of protein which they then can easily absorb.

This study aimed to evaluate the potential of blowfly maggots as an alternative dietary protein source for red tilapia by substituting blowfly maggot meal with fishmeal in a variety of experimental diets. The protein and amino acid composition of the diets was assessed and a feeding trial determined the effects of the diets on growth, feed efficiency and survival of the fish.

MATERIALS AND METHODS

Blowfly maggot meal preparation: Adult *C. megacephala* were collected from a local wet market (Petaling Jaya, Selangor, Malaysia) and kept in culture. Fresh beef liver was used as an egg-collecting medium. Maggots hatching from eggs over 4 days were killed with hot water, sieved, and oven dried at 100°C for 24 h before being ground into fine powder.

The protein content of blowfly maggot meal was determined using semi-micro Kjeldahl method (Helrich, 1990). Briefly, 12 ml of concentrated H_2SO_4 was used to digest 0.1 g of maggot powder for 1 h at 400°C in a fume hood using a BÜCHI Labortechnik K-435 digester. The digested product was distilled with NaOH for 5 min on an automatic rapid steam distillation machine (Gerhardt Vapodest 20). Crude protein content was estimated by multiplying the percentage nitrogen value with 6.25.

To determine amino acid composition, blowfly maggot meal was hydrolyzed with 6 N HCl at 100°C for 24 h. Amino acids were derivatized with phenylisothiocyanate to produce phenylthiocarbamyl amino acid derivatives. An internal standard containing a known amount of 17 common free amino acids was added to the hydrolyzed sample before filtering through a 0.2µm cellulose nitrate membrane. A reagent containing methanol, phenylisothiocyanate, triethylamine and H₂O in the ratio 7: 1: 1: 1 was allowed to react with the filtered sample for 20 min. This was followed by vacuum drying for 30 min. The sample was then dissolved in 0.1 M ammonium acetate (pH 6.5) before filtering (Merck Millipore Ltd.). 20 µl of sample was injected into a reversed-phase (Purospher STAR RP-18) column (5 mm) in a high performance liquid chromatography system (JASCO Md 2010) and monitored by UV absorption (Waters PicoTag). The chromatographic peak areas were identified and quantified. The composition of each amino acid in the sample was calculated by dividing the peak area of each by the internal standard in the chromatogram. This value is then multiplied by the total amount of internal standard added to the original sample.

Note that asparagine and glutamine are converted to aspartic acid and glutamic acid by acid hydrolysis so the values presented are for these molecules (Jabir *et al.*, 2012).

Experimental diets: Prior to formulation of the experimental diets, the proximate nutrient composition of fishmeal (obtained from Faculty of Agriculture, Universiti Putra Malaysia) and maggot meal was determined (following AOAC, 1990). Comparatively, maggot meal contains higher crude lipid and gross energy than fish meal (Table 1). Five experimental diets: a control diet (M0) containing fishmeal and no maggot meal and four diets with increasing levels of substitution of fishmeal by blowfly maggot meal were designed using feed formulation software (WinFeed v2.83, WinFeed Limited, Cambridge, UK). The diets were designed to contain approximately 30.0% of crude protein and 20.0 kJg⁻¹ gross energy (Table 2).

The diets were produced using a laboratory scale single screw extruder (Brabender KE19) from ingredients ground into fine powder, dried at 50°C for 12 h, sealed and stored at room temperature until use. Proximate nutrient composition analysis of the experimental diets followed AOAC (1990).

Feeding trial: Juvenile red tilapia were supplied by a local hatchery and quarantined for a week before the feeding trial. All fish were fasted for 24 h at the beginning of the trial and body weights were measured individually. Ten juveniles were randomly stocked into a glass tank ($60 \times 30 \times 30$ cm) equipped with a closed recirculation water system. Three tanks were set up for each experimental diet. Fish were kept in a natural photoperiod regime and the water temperature was $25\pm1.8^{\circ}$ C. The fish were fed twice daily for 60 days, at 0800 and 1600, at a daily feeding rate of 5% body weight.

At the end of the feeding trial, fish were fasted for 24 h before the final body weight was recorded. Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as:

SGR = ((ln (Final body weight) – ln (Initial body weight)) /number of days) X 100

 Table I: Proximate nutrient composition analysis of Menhaden fishmeal and blowfly maggot meal

Composition	Menhaden	Blowfly maggot
(g kg ⁻¹)	fishmeal	meal
Crude protein	587.0	555.0
Crude carbohydrate	N.A.	7.5
Crude lipid	96.0	254.0
Ash	190.0	13.0
Fiber	9.0	59.0
Gross energy (kJ g ⁻¹)	16.6	20.1

N. A. – Not available

Table 2: Composition of experimental diets

	M0	M25	M50	M75	M100
Ingredients (g kg ⁻¹)					
Fish meal	300	225	150	75	0
Maggot meal	0	75	150	225	300
Proximate Composition (g	kg-1)				
Crude protein	295.0	310.0	292.0	295.0	311.0
*Crude lipid	100	101	103	105	107
*Fiber	37.0	40.0	44.0	48.0	52.0
Gross energy (kJ g ⁻¹)	21.8	20.8	19.7	19.9	20.0
Essential amino acid (%)					
Arginine					1.02
Histidine					0.75
Isoleucine					1.11
Leucine					1.47
Lysine					1.59
Methionine					0.63
Phenylalanine					0.87
Threonine					1.41
Valine					1.05

* Value obtained with WinFeed v2.83.

Table 3: Crude protein (%) and amino acid composition of blowfly maggot meal (n=4) produced from maggots harvested I-4 days after hatching.

0				
Blowfly maggot		2	3	4
harvesting interval				
(day after hatching)				
Protein concentration	55.0±2.6ª	56.2±0.4ª	54.0±1.5 ^{ab}	52.4±0.2 ^b
Arginine*	3.4±0.5ª	2.7±0.0 ^{ab}	2.2±0.2 ^b	2.6±0.4 ^b
Histidine*	2.5±0.3 ^a	1.4±0.0 ^b	1.5±0.0 ^b	1.5±0.1 ^b
Isoleucine*	3.7±0.3ª	1.9±0.0 ^b	1.9±0.0 ^b	2.1±0.2 ^b
Leucine*	4.9±0.0 ^a	3.4±0.0 ^b	3.5±0.1 [♭]	3.7±0.4 ^b
Lysine*	5.3±0.3 ^a	4.1±0.2 ^b	4.4±0.2 ^{bc}	4.6±0.4°
Methionine*	2.1±0.5 ^a	1.2±0.0 ^b	1.3±0.0 ^b	1.5±0.1 ^b
Phenylalanine*	2.9±0.4 ^{ab}	2.5±0.0 ^a	3.2±0.0 ^b	4.0±0.4°
Threonine*	4.7±0.1ª	2.7±0.0 ^b	2.3±0.2 ^b	2.3±0.3 ^b
Valine*	3.5±0.3 ^a	2.3±0.0 ^b	2.4±0.1 ^b	2.6±0.3 ^b
Alanine	3.9±0.1ª	3.1±0.1 ^b	2.8±0.1 ^b	2.8±0.3 ^b
Aspartic acid	12.1±2.2ª	10±0.0ª	10.7±1.9ª	12.1±2.4ª
Cystine	2.9±2.9 ^a	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
Glutamic acid	15.7±1.6ª	13.2±1.6ª	13.8±1.4ª	14.4±2.0 ^a
Glycine	3.2±0.0 ^a	2.4±0.0 ^b	2.4±0.0 ^b	2.3±0.2 ^b
Proline	3.7±0.6 ^a	2.0±0.1 ^b	2.0±0.1 ^b	2.2±0.2 ^b
Serine	2.9±0.2ª	2.0±0.0 ^b	2.0±0.1 ^b	2.2±0.2 ^b
Tyrosine	3.2±0.5ª	1.8±0.0 ^b	2.9±0.0 ^a	4.2±0.4 ^c
*Eccontial amina acid	Maan+CD	within a ra	w followed	by different

*Essential amino acid. Mean±SD within a row followed by different letters are significantly different (P<0.05).

FCR = Total feed intake (g) / (Final body weight – Initial body weight) (g)

PER = (Final body weight – Initial body weight) (g) / Total protein intake (g)

Survival was calculated as follows:

Survival rate = (number of fish alive at end of feeding trial / number of fish at beginning of feeding trial) X 100

All the data were recorded as mean±SD and were subjected to one-way analysis of variance (ANOVA). All percentage data were arcsine transformed prior to analysis. The differences among means were analyzed using Duncan's multiple range test.

RESULTS

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Crude protein and amino acid composition of blowfly maggot meal: Blowfly maggot meal derived from maggots harvested 1 day after hatching had the highest concentration of amino acids compared to the other meals, but all were very similar (all differing by <2.0%), with the exception of cystine (Table 3). Crude protein concentration was also very similar (average 54.4%) (Table 3).

Growth, feed efficiency and survival of juvenile red tilapia: The initial body weights of the juvenile red tilapia were all similar (~3.00 g) (Table 4). The final body weight, weight gain and SGR, measured at the end of the 60 days trial, showed a direct relationship with the percentage of maggot meal in the experimental diet (Table 4), with the largest values found in the fish fed with the M100 diet (i.e. 100% maggot meal, no fishmeal). Fish fed on the M100 diet also showed the best (lowest) FCR (1.34) and (highest) PER (0.30), although there were no significant differences among the experimental diets for these measures (Table 4). The highest survival rate was also observed for tilapia fed the M100 diet but no significant differences (P>0.05) were observed among the treatments due to large standard deviations.

DISCUSSION

Fishmeal is an important protein source in aquafeed. The cost of fish farming is expected to rise as the demand for fishmeal increases while its availability is likely to remain constant or decrease. Various studies have been conducted with sustainable alternative protein sources to determine their effects on fish growth (Silva *et al.*, 2010; Cabral *et al.*, 2011) and these have shown contradicting results.

Housefly (Musca domestica) maggot meal was reported to contain 39-65% (Atteh and Ologbenla, 1993; Awoniyi et al., 2003), while the protein content of Chrysomya megacephala maggot meal, reported here, ranged from 52-56% depending on the age of maggots at harvesting. Such variations in protein content could be attributed to the processing, drying, storage and protein estimation methods employed, or the media used for the production of housefly maggots (Awoniyi et al., 2003; Ogunji et al., 2008). Crucially, the values for maggot meal are similar to those of locally produced fishmeal (58.7% for Menhaden fishmeal). Proximate analysis of Menhaden fishmeal and blowfly maggot meal suggested crude lipid was higher in maggot meal (254 g kg⁻¹), a finding consistent with a previous study (233 g kg⁻¹) (Ogunji et al., 2008) where the nutrient composition of housefly maggot meal was evaluated.

High levels of fishmeal replacement with other animal or plant proteins have frequently led to growth reduction of fish (Begum *et al.*, 1994; Ogunji *et al.*, 2007; Cabral *et al.*, 2011). This phenomenon is usually related to a deficiency or absence of one or more essential amino acids in those animal and plant protein sources. Moreover, insufficient amounts of certain essential amino acids in any given diet can cause fish to suffer cataracts (methionine and tryptophan) and scoliosis (tryptophan) (Cowey, 1994).

Table 4: Body weight (BW), special growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) of red tilapia Oreochromis sp. after feeding with the experimental diets for 60 days and the initial body weight.

0 1	,	, ,				
Experimental diets	M0	M25	M50	M75	M100	
Initial BW (g)	2.75±0.57 ^a	3.00±0.27 ^a	3.21±0.22 ^a	3.29±0.2ª	3.11±0.14ª	
Final BVV (g)	5.64 ± 0.93 ^a	6.34±1.71ª	7.47±1.44 ^a	8.53±2.22 ^{ab}	10.63±2.65 ^b	
SGR (% day ⁻¹)	1.21±0.3ª	1.21±0.37ª	1.39 ±0.21ª	1.55±0.34 ^{ab}	2.02±0.27 ^b	
FCR	2.89±0.99 ^a	2.63±1.10ª	2.08±0.35 ^a	1.97±0.50 ^a	1.34±0.14ª	
PER	0.18±0.06 ^a	0.17±0.06ª	0.20±0.05 ^a	0.21 ± 0.07^{a}	0.30±0.12ª	
Survival rate (%)	63.33±11.55ª	60.00 ± 10.00^{a}	76.67±15.28ª	73.33±25.17ª	80.00±26.46 ^a	
Maan + SD within a new fallowed by different letters are similiantly different (D<0.05)						

Mean±SD within a row followed by different letters are significantly different (P<0.05).

High levels of fishmeal replacement with housefly maggot meal have been associated with low body weight gain in both fish and chickens (Oyelese, 2007; Ogunji et al., 2008). Earlier studies indicated that housefly maggot meal should only partially substitute fishmeal in the diets of omnivorous fish species such as catfish and Nile tilapia (Oyelese, 2007; Ogunji et al., 2008). Some authors reported replacement of fishmeal with housefly maggot meal at 50% or less provided the optimum level in chicken feed (Awoniyi et al., 2003; Adenji, 2007). These earlier studies contrast with the present study which showed increased substitution of fishmeal by blowfly maggot meal improved the growth, survival and feed efficiency of juvenile tilapia with the total replacement diet giving the optimal results. Although palatability of the maggot meal was not directly tested, these results and our observations in the lab indicated that there was no rejection by the fish.

Conclusion: Blowfly maggot meal contained all the essential amino acids needed by juvenile tilapia for normal growth, and equivalent protein content to fishmeal. Diets with increased substitution of fishmeal by blowfly maggot meal improved the growth, feed efficiency and survival of juvenile tilapia with the total replacement diet giving the optimal results. Blowfly maggot larvae can be mass-produced in a short period of time (less than one week) from agricultural waste and replacement of fishmeal with blowfly maggot meal in tilapia feed should directly reduce the production costs. Further studies should be conducted to improve and refine maggot meal as a component feed for other commercially important fishes.

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