Growth performance, feed utilization and body composition of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) fed with different levels of black soldier fly, *Hermetia illucens* (Linnaeus, 1758) maggot meal diet

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Abstract

In this study, fish meal (FM) was replaced by the black soldier fly maggot meal (BSFM) with replacement levels at 0%, 25%, 50%, 75% and 100%. The feeding trial was conducted for 56 days and the effect of each replacement level on the growth performance, feed utilization, body composition and survival of the experimental fish was assessed. All the experimental diets were well accepted by the fish. No mortality was observed during the experimental period. Diet 3 resulted in the highest weight gain and SGR values of 8.74±0.18 and 2.43±0.04% respectively. FCR and PER values obtained for Diet 3 were also better compared to that with other diets. Although there were no significant differences in crude protein content among fishes fed different diets (Diet 1 to Diet 5), fish fed Diet 3 showed significant (p<0.05) increase in crude protein content at the end of the experiment. Based on these results, it may be concluded that BSFM can be used to replace FM with up to 50% replacement without causing adverse effects on growth and feed utilization parameters.

Keywords: *Hermetia illucens*, Black soldier fly maggot meal, *Oreochromis niloticus*, Growth performance, Feed utilization

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Introduction

Extensive research has been done to seek alternative protein sources to replace fish meal in the fish feed industry. Aquaculture can no longer depend on fish meal as a sole protein source because of its fluctuating supply and increasing price trend. The increase in price of fish meal in the market will indirectly increase the production cost of the fish farmed (Hardy, 2010). Several types of feed have been investigated in an attempt to seek a replacement for fish meal in fish feed (Lunger *et al.,* 2007; Silva *et al.,* 2010; Cabral *et al.,*2011; Richard *et al.,* 2011; Muin *et al.,* 2013; Muin *et al.,* 2014; Muin *et al.,* 2015). These feed include animal protein, plant, and also aquatic plant-based diet. One of the criteria in order to be a good alternative protein source is the ability to supply adequate protein with appropriate balance in amino acid content required by fish to support growth and health (Khan *et al.,* 2012; Naz and Javed, 2013). Furthermore, these feeds should be cheaper than fish meal, easy to access, and found in abundance locally.

Recently, researchers tend to use insect-based meal as an alternative to replace fish meal protein. A few species of insects have been studied. For example, Jabir *et al.,* (2012a, 2012b) and Jabir *et al.,* (2014) evaluated the potential of super worm meal, Awoniyi *et al.,* (2003); Fasakin *et al.,*(2003); Ajani *et al.,* (2004); Ogunji *et al.,* (2007) and Atteh and Ologbenla (2015) worked on housefly maggot meal, Taufek *et al.,* (2016) evaluated the potential of black cricket meal in African catfish diet, and Sing *et al., (*2014) evaluated and analyzed the protein of blow fly maggot meal in tilapia diet.

The black soldier fly (BSF) is a non-pest tropical and warm-temperate region insect and can colonize a wide variety of decomposing vegetables and animal matter. It is a member of the Stratiomyidae family and is not considered as a pest as the adult flies are not attracted to human habitation or food and therefore do not transmit diseases (Furman *et al.,* 1959). These pre pupae contain 42% crude protein and 35% fat which makes it a suitable source for fishmeal replacement in commercial fish feed production and also as a promising source for biodiesel due to its high fatty acid content (Newton *et al.,* 1977). However, information about Black soldier fly maggot meal (BSFM) in fish feed is still limited.

The purpose of this study is to evaluate the use of the black soldier fly (*Hermetia illucens*) maggot meal (BSFM) protein source as a fish meal substitute in the practical commercial tilapia diets and its relation to growth performance, feed utilization, and body composition.

Materials and methods

BSF was cultured at the AquaNutriBiotech Research Laboratory, University of Malaya, Malaysia. After 2 weeks of feeding,

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matured larvae were collected and sacrificed humanely by putting them into a freezer before being dried at 50°C for 24 hours. Dried larvae was then ground into a powder and kept in a cold room at 4°C. BSFM and all the raw materials were analyzed for their chemical content using standard methods according to AOAC (2002) (Table 1). Five different feed levels of FM replacement were formulated using WinFeed (version 2.8) software based on isonitrogenous crude protein (30%) content. The protein replacement was at 0% (Diet 1), 25% (Diet 2), 50% (Diet 3), 75% (Diet 4) and 100% (Diet 5). All experimental diets were prepared using the 'Mini Pelleting Machine' to produce standard sized pellet of 1.5 mm in diameter. Every diet was then dried in an oven at 60 C for 24 hours to prevent fungus infestation. Samples from each diet were then chemically tested for its moisture, protein, lipid, fiber and ash. The results are shown in Table 2.

Feeding trials were conducted for eight weeks. The fish were obtained from a commercial supplier in Balakong, Selangor, Malaysia. Prior to the feeding trials, the fish were acclimatized for a week and were fed with commercial pellet. The fish were then randomly chosen and weighed, before being randomly distributed into fifteen plastic tanks. Each tank was stocked with ten fish. Each diet was assigned to triplicate tanks. Several fish were frozen in the freezer for proximate analysis. The feeding level was at 5% of the fish body weight. Fish were fed two times daily. The fish were weighed once in two weeks and the appropriate feeding ration was adjusted for the following week. At the start of the experiment, ten fish were sacrificed and kept frozen till the end of the experiment for proximate analysis. At the end of the experiment, all the fish were sacrificed, pooled, and analyzed for their nutrient composition.

Proximate composition of BSFM, fish meal, diet samples, fish carcasses before and after experiment were determined using standard laboratory methods according to AOAC (2002). Crude protein content was determined using automatic Kjeldahl technique and multiplying N by 6.25 factors. Crude lipid was evaluated by Soxtec Tecator System and dry matter was calculated from weight loss after 24 hours drying at 100 C. For ash, the sample was burnt in an oven at 600 C until constant weight was achieved. Fiber analysis was done using the fiber cap procedure which includes digestion by alkali and acid. Amino acid analysis was done using the HPLC (Jasco, CO-2056 Plus, Intelligent Column Oven) by comparing the peak retention time to a known standard.

Growth performance was measured in order to analyze the feed utilization efficiency of the fish.

Body weight gain (BWG) = $W_t - W_i$

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

Specific growth rate (SGR) = $[(\ln W_t - \ln W_i)/T] \times 100$

Protein efficiency ratio (PER) = wet weight gain (g) / total protein intake (g)

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Survival rate (%) = (final number of remaining fish / initial number of fish)× 100

Where W_t – mean final weight,

W_i– mean initial weight,

T – feeding trial period in days,

Analyses were performed using the statistical package SPSS 14.0. All the data were subjected to one-way analysis of variance (ANOVA) followed by a comparison of means using the Duncan Test. All differences were regarded as significantly different at $p<0.05$ among treatment groups.

Results

From Table 1, BSFM used in this study contain 41.74±1.09% crude protein and 28.74±1.44% lipid. Besides that, the proximate analysis and amino acid content of experimental diets are shown in Table 2 and Table 3.

Table 4 shows the growth performance of tilapia fingerlings fed experimental diets. Initial stocking density of fish was ten fish per aquarium tank. At the end of the experiment, there were no significant differences ($p>0.05$) in terms of survival rate of the fish fed five different diets. All experimental diets show 100% survival rate where all the fish survived and no mortality was recorded. The growth of fish was obtained by measuring the weight gain every two weeks. The initial mean weight of fish fed diets 1, 2, 3, 4 and 5 were 3.07± 0.09 g, 3.09 ± 0.04 g, 3.03 ± 0.05 g, 3.02 ± 0.07 g and 2.99 ± 0.02 g, respectively as shown in Table 4. At the end of the experiment, the final weight of fish fed diet 2 was the highest (11.83 ± 0.44), followed by diet 3 (11.77 ± 0.16 g), diet 4 (11.43± 0.33g), diet 1 (10.86 ± 0.55 g) and diet 5 (10.43 ± 0.28 g**).**

No significant differences ($p>0.05$) were observed for specific growth rate (SGR) and feed conversion ratio (FCR) among all diets. However, the fish fed diet 3 showed the highest SGR (2.43±0.04%) and the best FCR (2.91±0.10) compared to that in other diets. On the other hand, weight gain and protein conversion ratio (PER) showed significant differences ($p<0.05$) among all diets. For weight gain (WG) and the protein efficiency ratio (PER), the fish fed with diet 3 produced the best results at 8.74±0.18g and 1.17±0.04 respectively.

There were significant differences ($p<0.05$) for crude protein content in muscle tissue among fish in all treatments as shown in Table 5. The initial body composition for crude protein content of the fish was 51.03 ± 0.22%. Diet 2 (25% replacement of fish meal) resulted in the highest increment in crude protein level (60.88±2.75), followed by fish fed diet 5 (58.75 ± 0.84), diet 3 (58.73 ± 0.28), diet 4 (58.44 ± 1.09) and diet 1 (58.16 ± 0.29).

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Table 1: Proximate composition of BSFM

Chemical composition (%)	Composition (mean ± SE)
Dry matter (%)	89.37 ± 0.12
Crude Protein (%)	41.74 ± 1.09
Crude lipid (%)	28.74 ± 1.44
Ash (%)	10.64 ± 0.04
Fiber (%)	12.50 ± 0.23

Table 2: Formulation and chemical composition of the experimental diets.

Ingredients	Diet 1 (0%)	Diet 2 (25%)	Diet 3 (50%)	Diet 4 (75%)	Diet 5 (100%)
Fish meal	30	22.5	15	7.5	0
BSF meal	0	7.5	15	22.5	30
Soy bean meal	9.4	15.33	21.26	27.26	33.12
Rice bran	43.6	37.67	31.74	25.81	19.88
Corn meal	15	15	15	15	15
Vitamin[a]	0.5	0.5	0.5	0.5	0.5
Mineral[b]	0.5	0.5	0.5	0.5	0.5
Dcp	1	1			
Total	100	100	100	100	100
Nutrient levels determined by analysis, % (as basis)					
Dry matter	92.09	91.52	90.79	91.34	91.61
Crude protein	30.42	29.76	29.28	29.65	29.72
Crude fat	6.97	8.60	10.18	11.33	12.27
Crude ash	12.71	11.09	9.65	8.19	6.29
Crude fiber	3.02	3.14	4.00	4.73	5.83
NFE[c]	38.96	38.93	37.68	37.45	37.51
Gross energy (kJ/g)[d]	16.90	17.39	17.68	18.19	18.59

[a]vitamin A 50.000MTV; Vitamin D3 10.000MTV; Vitamin E 75.000gm; Vitamin K3 20.000gm; vitamin B1 10.000gm; vitamin B2 30.000gm; vitamin B6 20.000GM; Vitamin 12 0.100gm; calcium D-Pantathenate 60.000gm; nicotinic acid 200.000gm; folic acid 5.000gm; biotin 235.000gm.

[b]selenium 0.200gm; iron 80.000gm; manganese 100.000gm; zinc 80.000gm; copper 15.000gm; potassium chloride 4.000gm; magnesium oxide 0.6000gm; sodium bicarbonate 1.500gm; iodine 1.000gm; cobait 0.250 gm.

[c]NFE = 100 – (% protein + % lipid + % ash + % fiber+ % moisture)

[d]Gross energy (GE) was calculated using the following factors: crude protein= 23.9kJ/g, crude lipid= 39.8 kJ/g and NFE = 17.6 kJ/g (Schulz *et al*., 2005).

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Table 3: Essential amino acid composition of diets used in this study (mg/crude protein).

Amino acids	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Histidine	7.52±0.40^{ab}	7.70±0.17^{b}	7.70±0.03^{b}	7.49±0.03^{ab}	6.91±0.10^{a}
Arginine	18.99±0.09^{b}	17.64±0.04^{ab}	18.34±0.18^{ab}	16.67±0.31^{a}	17.35±0.21^{ab}
Threonine	11.87±0.02^{a}	12.61±0.11^{a}	12.07±0.26^{a}	12.19±0.15^{a}	11.87±0.93^{a}
Valine	14.31±0.19^{a}	15.32±0.24^{b}	15.69±0.10^{bc}	15.99±0.01^{cd}	16.45±0.04^{d}
Methionine	6.72±0.07^{dc}	5.89±0.01^{cd}	5.42±0.01^{bc}	4.79±0.03^{ab}	4.40±0.02^{a}
Isoleucine	11.86±0.16^{a}	12.50±0.08^{b}	12.88±0.10^{bc}	12.90±0.01^{bc}	13.26±0.08^{c}
Leucine	21.95±0.05^{a}	22.29±0.06^{ab}	22.72±0.05^{b}	22.50±0.30^{b}	22.72±0.01^{b}
Phenylanine	14.00±0.16^{b}	13.08±0.13^{a}	14.85±0.01^{cd}	15.20±0.08^{d}	14.38±0.01^{bc}
Lysine	18.80±0.12^{c}	18.85±0.07^{c}	18.18±0.05^{b}	16.86±0.03^{a}	16.65±0.19^{a}

All values are means ± SE for triplicate feeding groups and values in same row with different letters are significantly different (p<0.05).
Essential amino acid requirements of Nile tilapia according to National Research Council (1993) are: tryptophan 1.00, lysine 5.12, histidine 1.72, arginine 4.20, threonine 3.75, valine 2.80, methionine 2.68, isoleucine 3.11, leucine 3.39, phenylalanine + tyrosine 3.75.

Table 4: Growth performance of tilapia fingerling fed with experimental diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Weight, g	3.07 ± 0.09^{a}	3.09 ± 0.04^{a}	3.03 ± 0.05^{a}	3.02 ± 0.07^{a}	2.99 ± 0.02^{a}
Final weight, g	10.86 ± 0.55^{ab}	11.83 ± 0.44^{b}	11.77 ± 0.16^{b}	11.43 ± 0.33^{ab}	10.43 ± 0.28^{a}
Weight gain, g	7.79±0.52^{ab}	8.74±0.43^{b}	8.74±0.18^{b}	8.41±0.26^{ab}	7.44±0.29^{a}
SGR, %	2.25±0.09^{a}	2.39±0.06^{a}	2.43±0.04^{a}	2.38±0.02^{a}	2.23±0.06^{a}
FCR	3.18±0.13^{a}	3.08±0.21^{a}	2.91±0.10^{a}	3.09±0.04^{a}	3.31±0.08^{a}
PER	1.04± 0.04^{ab}	1.10± 0.07^{bc}	1.17 ± 0.04^{c}	1.09± 0.01^{abc}	1.02± 0.02^{a}
Survival, %	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

SGR = (lnW2 – ln W1/T) X 100, FCR = food fed/ live weight gain, PER = live weight gain (g)/ protein fed (g)
All the values are means ± SE for triplicate feeding groups. Means on the same row with different letters are significantly different (p<0.05).

Table 5: Initial and final body composition of tilapia fingerlings fed on experimental diets (%).

Component	Initial	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Dry matter	99.20±0.24^{a}	96.24±0.05^{a}	96.03±0.09^{a}	95.76±0.36^{a}	96.15±0.25^{a}	95.86±0.25^{a}
Protein	51.03±0.22^{a}	58.16±0.29^{b}	60.88±2.75^{b}	58.73±0.28^{b}	58.44±1.09^{b}	58.75±0.84^{b}
Fat	10.82±0.16^{a}	21.02±0.43^{c}	17.95±1.42^{b}	21.07±0.45^{c}	21.02±0.43^{c}	19.96±0.48^{bc}
Ash	7.50±0.11^{a}	15.92±0.43^{bc}	16.51±0.49^{c}	15.81±0.64^{bc}	14.80±0.11^{b}	15.58±0.41^{bc}

All the values are means ± SE for triplicate feeding groups. Means on the same row with different letters are significantly different (p<0.05).

Discussion

From this study, it can be shown that BSFM contain 41.74±1.09% crude protein and 28.74±1.44% lipid. The value for crude protein is higher than the values reported by other researchers previously. Studies done by Sheppard *et al.*, (1994) and Newton *et al.*, (2005) reported that the prepupae of black soldier fly contained approximately 40% protein and 30% fat. Ojewola *et al.*, (2005) reported that factors such as

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source, stage of harvesting, methods of processing and drying may affect the nutrient content of samples. Furthermore, Jabir *et al.,* (2012b) working on Super worm meal reported that feed consumed by the super worm during its growth affect the crude lipid content.

At the end of this experiment, all fishes in all treatments showed good survival. This indicates that the pelleted diet was highly palatable and well accepted by the fish. Rodríguez-Serna *et al.,* (1996) said that unacceptability due to the unpalatability of the diets fed to fish is one of the most common problems when an alternative protein source was used to replace FM. Besides that, good handling practices and water quality management during the experimental period may affect the survival rate of fish (Bichi and Ahmad, 2010).

In the present study, there are slightly significant differences in terms of weight gain among all diets. Weight gain of fish fed Diet 2 and Diet 3 was significantly higher compared to that in other treatments. However, weight gain of experimental fishes decreased as the replacement of FM increased further. This indicates that tilapia fish could accept the alternative protein (BSFM) optimally only up to the 50% inclusion level. SGR, There were no significant differences recorded for SGR among all treatments. These results indicate that replacement of fish meal with BSFM up to 100% did not affect the specific growth rate of fish. The best SGR values recorded was fish fed Diet 3 with a value of 2.43±0.04%.

There was no significant difference among all diets in terms of FCR value. However, Diet 3 (2.91±0.01) produced the best value compared to others. A low value of FCR is a good indicator of a high quality feed. This result suggests that 50% replacement of fish meal by BSFM could be used to feed Nile Tilapia fingerlings in order to obtain good feed utilization. However, St-Hilaire *et al.,* (2007) reported that good FCR was obtained in fish fed rainbow trout diets replaced with 25% black soldier fly meal without causing adverse effects on the FCR of the fish. In this study, as the percentage of the replacement of fishmeal increases, the FCR values also increases. It is unknown why this happens and needs further investigation. However, St-Hilaire *et al.,* (2007) assumed that diets containing more BSFM contained chitin and this may affect their digestibility. Similar findings were reported by Köprücü and Özdemir (2005) who found that lower ADCs dry matter, protein, average amino acid, lipid and energy were observed in tilapia fed feeds with high content of ash and chitin. However, they did not provide the digestibility data to support their hypothesis as it was not collected.

PER is an indicator of quality of protein content in feedstuff and usually high PER values are required for better feed utilization of fish. In the present study PER values ranged from 1.02±0.02 to 1.17±0.04 which were lower than those reported by other

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researchers working with tilapia. In contrast to the present study, higher PER values were reported Maina *et al.,* (2002) and Ogunji *et al.,* (2008) for tilapia fed a diet with FM replaced by maggot meal. The differences between PER reported by the different studies could be due to quality of dietary protein used**.**

For the whole body protein composition, there are slightly significant differences between initial carcass and final carcass in fish fed with different level inclusion of FM. However the body protein in all experimental groups did not differ much during the experimental period. These findings showed that the whole body protein content of fish was not clearly affected by the diet composition used. This could be due to the duration of the trial which was limited to only 8 weeks; therefore limiting the ability of the study to detect small differences in these parameters. However for fat content, it shows large variations. Similar results were obtained by Atack *et al.,* (1979) and Hasan and Macintosh (1993). They reported that lipid content tends to show greater fluctuations than other carcass components.

In conclusion, the present study indicates that BSFM can replace up to 50% (Diet 3) of the protein in FM without adverse effects in growth performance, feed utilization and body composition of tilapia. However, further studies on palatability and digestibility should be conducted to strengthen the findings of the present study.

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