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ORIGINAL RESEARCH

Efficacy of insect larval meal to replace fish meal in juvenile barramundi, *Lates calcarifer* reared in freshwater

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Abstract The present experiment was conducted to evaluate the efficacy of dietary protein from black soldier fly, *Hermetia illucens,* larval meal (BSFL) to replace fish meal (FM) protein in juvenile barramundi, *Lates calcarifer*. Larvae of black soldier fly were fed with the underutilised crop, sesbania, *Sesbania grandiflora.* Five isonitrogenous (44% crude protein) and isocaloric (16.0 kJ available energy/g) experimental diets were formulated to replace FM using processed BSFL meal at 0 (control), 25% (BSFL25), 50% (BSFL50), 75% (BSFL75) and 100% (BSFL100). Data for proximate and amino acid analysis suggested BSFL meal as an inferior protein ingredient than FM, but parallel to soybean meal. At the end of 8 weeks of fish feeding trial, there were no significant differences in the average weight gain (WG) and specific growth rate among the group of fish-fed control, BSFL25 and BSFL50 diets ($P < 0.05$). Although numerical differences were recorded in the fish whole-body proximate composition, crude protein and moisture content were not much affected by the different dietary treatments. Essential amino acids including arginine, histidine, lysine and methionine were found to be higher in the whole body of fish-fed BSFL100 diet. Broken line regression analysis of average WG showed an optimum FM replacement level of 28.4% with BSFL meal. Therefore, the present experiment clearly demonstrates that the maximal dietary inclusion level of BSFL meal as FM protein replacer for the optimum growth of juvenile barramundi reared in freshwater could be greater than 28.4% but less than 50%, without any adverse effects on the fish whole-body proximate and amino acid composition.

Keywords Black soldier fly · Fish meal · Growth · Amino acids · Barramundi

Introduction

Aquaculture has become the fastest growing food-producing industry around the world; however, the production efficiency of aquaculture could be further increased through the development of nutritious and cost-effective alternatives to traditional and finite marine protein feedstuffs such as fish meal (FM) (Suarez et al. 2013; Katya et al. 2014). Numerous studies have been carried out to replace or reduce the inclusion level of FM and to identify promising alternative protein sources in aquafeeds. Some of the thoroughly investigated ingredients to partially replace FM in fish feed include plant protein sources such as soybean meal, corn gluten

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meal and soy protein concentrate, by-products of mushroom (Carter and Hauler 2000; Refstie et al. 2001; Choi et al. 2004; Lim et al. 2004; Kim et al. 2008; Katya et al. 2014) and animal protein sources such as meat and bone meal, blood meal, feather meal, poultry by-product meal and lysine by-product (Bai et al. 1997, 1998; Lee and Bai 1997a, b). However, least attention has been given to access the use of unconventional but promising feedstuffs such as insect meal in commercial fish feed formulation.

In last few years, a considerable number of researches have shed the light on the efficacy of dietary insect meal in several aquaculture species including tilapia, *Oreochromis niloticus,* salmon, *Salmo salar*, African catfish, *Clarias gariepinus,* turbot, *Psetta maxima,* and rainbow trout, *Oncorhynchus mykiss* (Kroeckel et al. 2012; Fasakin et al. 2003; Lock et al. 2015; St-Hilaire et al. 2007a, b; Ogunji et al. 2007, 2008; Rana et al. 2015) feeds. Among a number of promising insect species, farming of black soldier fly, *Hermetia illucens* larvae/pre-pupae has received top priority for the commercial exploitation (Lock et al. 2015). The overall nutrient content viz. crude protein, lipid and essential amino acid content of insect meal closely resemble with FM, and the pre-pupae meal have been demonstrated to be suitably alternative for FM (Kroeckel et al. 2012; Lock et al. 2015; Rana et al. 2015) in several aquaculture species.

Barramundi or Asian sea bass, *Lates calcarifer* is one of the commercially important fish species widely cultured in several Asian countries. Nutrient requirements for this species has been well defined, considerable efforts have also been made in the three decades to develop nutritionally balanced and cost-effective diets (Glencross 2003). The commercial feed formulation for this species heavily depends upon the FM as the primary protein source. Although, a number of FM alternative ingredients of plant and animal origin have been investigated (Boonyaratpalin et al. 1998; Glencross 2003), use of locally available but nutritionally efficient feedstuff in barramundi feeds is warranted. Therefore, the present experiment was conducted to evaluate the replacement efficacy of protein from BSFL meal with FM protein in juvenile barramundi, reared in freshwater.

Materials and methods

Insects rearing and larval meal production

The insectarium and larval rearing units were set up at the field research center of Crops for The Future (CFF), Kuala Lumpur, Malaysia. Based upon the experience on behaviors of adult insects as well as using the knowledge on the life cycle of black soldier fly, a calm place was selected and natural environment were simulated to attract and induce the mating of adult insects. Six small plastic buckets filled with banana leaves and hay were hanged around the corners and center of the insectarium to collect insect eggs every morning. A series of round bottom 500 L capacity plastic tanks were filled one-third with the blend of underutilized crop sesbania, *Sesbania grandiflora*. Self-harvesting larvae collection chambers were prepared using flat wooden sheet fixed at 45° angle opening outside the plastic tanks. The BSFL production method were similar that is used by Ajani et al. (2004) and Adesulu and Mustapha (2000) for housefly maggot meal.

Experimental design and diets

Formulation and proximate composition of the experimental diets is shown in Table 1. Five isonitrogenous (44% crude protein) and isocaloric (16.0 kJ available energy/g) diets were formulated to replace fish meal using BSFL meal at 0 (control), 25% ($BSFL_{25}$), 50% ($BSFL_{50}$), 75% ($BSFL_{75}$) and 100% ($BSFL_{100}$). Fishmeal, soybean meal, corn gluten meal and squid liver powder served as the major protein sources in the experimental diets; fish oil was used as the lipid source while dextrin flour was the carbohydrate source. Fish meal was substituted at graded level and accordingly adjustments were done in other ingredients to balance the protein and energy content in all experimental diets. Feeds were designed and formulated to meet the optimum nutrient requirement of experimental fish, following the nutrient requirements extensively reviewed and recommended for juvenile size barramundi (Glencross 2003).

Procedures for diet preparation and storage were as previously described by Bai and Kim (1997). After thoroughly mixing the dry ingredients and fish oil with 30%-filtered tap water, experimental diets were pelleted with a laboratory pelleting machine without heating, using a 2-mm diameter module (MK-NG 1500

Table 1 Formulation and proximate composition of the experimental control diet (% dry matter base)

Ingredients	Control	$BSFL_{25}$	$BSFL_{50}$	$BSFL_{75}$	$BSFL_{100}$
Fish meal	40	30	20	10	0
BSF meal	0	15.4	30.8	46.2	61.6
Dextrin	16.4	12.3	8.2	4.1	0
Soybean meal	12	12	12	12	12
Corn meal	5.6	5.7	5.8	5.9	6.0
Squid liver powder	15	15	15	15	15
Fish oil	6.0	4.6	3.2	1.8	0.4
Vitamin premix[a]	3	3	3	3	3
Mineral premix[b]	2	2	2	2	2
Proximate composition[c]					
Crude protein	42.0	43.1	42.8	42.4	41.8
Crude lipid	12.0	11.8	12.1	12.0	12.4
Moisture	9.2	9.7	10.1	9.1	9.7
Crude ash	10.2	10.1	10.4	10.2	10.3

Diets represent; control (without black soldier fly larval meal inclusion), BSFL25 (25%), BSFL50 (50%), BSFL75 (75%) and BSFL100 (100% fish meal replacement using black soldier fly larval meal)

[a](as mg/kg in diets): ascorbic acid, 300; dl-calcium pantothenate, 150; choline bitatrate, 3000; inositol, 150; menadione, 6; niacin, 150; pyridoxine·HCl, 15; riboflavin, 30; thiamine mononitrate, 15; dl-α-tocopherol acetate, 201; retinyl acetate, 6; biotin, 1.5; folic acid, 5.4; B12, 0.06

[b]Contains (as mg/kg in diets): NaCl, 437.4; MgSO4·7H2O, 1379.8; NaH2P4·2H2O, 877.8; Ca(H2PO4)2·2H2O, 1366.7; KH2PO4, 2414; ZnSO4·7H2O, 226.4; Fe-citrate, 299; Ca-lactate, 3004; MnSO4, 0.016; FeSO4, 0.0378; CuSO4, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; NaSeO3, 0.00025

[c]Values are average from duplicate groups of samples

WSL food grinder). All the feeds were air-dried for 24 h and after processing, all diets were kept at −4 °C in the cold storage until use.

Experimental fish and feeding trial

Juvenile size barramundi, *Lates calcarifer* fish acclimated to freshwater were obtained from the hatchery Biodesaru, Gelang Patah Johor, Malaysia. Fish were transported to the experimental station (FishPLUS laboratory, FRC, Crops for The Future) and acclimated to the experimental conditions for 2 weeks before the feeding trial began. During the acclimation period, fish were fed control diet twice daily (10:00 and 1800 h) at approximately 3% of wet body weight/day. Twenty barramundi fish averaging 6.7 ± 0.1 g (mean ± standard deviation) were randomly distributed in fifteen individual fish groups to each of fifteen aquaria. Each aquarium was then randomly assigned to one of three replicates of five experimental diets. Triplicate groups of fish were fed one of the experimental diet twice daily (10:00 and 17:00 h) at approximately 2% of wet body weight/day at the beginning and 3% of wet body weight/day at the end of the feeding trial for 8 weeks. Total fish weight in each aquarium was determined every 2 weeks, and the amount of feeds fed to the fish was adjusted accordingly.

The feeding trial was conducted in an indoor semi-recirculation system with fifteen 300 L of aquaria receiving filtered freshwater from the center tank. Supplemental aeration was provided to maintain dissolved oxygen levels near 6.5 ± 0.5 (mean ± SD) ppm. Water temperature was 24 ± 1 °C (mean ± SD); pH was 7.5 ± 0.3 (mean ± SD) and the photoperiod of 12:12 (light: dark) was maintained throughout the experimental period.

Sample collection and analysis

At the end of the feeding trial, all of the fish were weighed and counted for the calculation of weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate. Growth performance parameters including WG, SGR and FCR were calculated using formulas.

$$\text{Weight gain } (\%) = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100.$$

$$\text{Specific growth rate } \left(\% \text{ day}^{-1}\right) = [(\log_e \text{final wt.} - \log_e \text{initial wt.})/\text{days}] \times 100.$$

$$\text{Feed conversion ratio} = \text{feed}/\text{wet weight gain}.$$

Protocol for the feeding trial, fish anesthesia, sample collection and analysis were followed as prescribed and approved by the Animal ethics committee, University of Nottingham. After the final weighing, five fish were randomly collected from each aquaria and frozen at −20 °C for analysis. The proximate composition and amino acid analysis of the experimental diets, ingredients and fish whole body was performed by the standard methods of AOAC (1995). Samples were dried to a constant weight at 105 °C to determine moisture content. Ash was determined by incineration at 550 °C, crude lipid by soxhlet extraction using a Soxtec system 1046 (Tecator AB, Hoganas, Sweden), and crude protein by Kjeldahl method (N × 6.25) after acid digestion. Amino acid analysis was carried out using Ninhydrin method (Sykam Amino Acid Analyzer S433; Sykam, Eresing, Germany).

Statistical analysis

All data were analyzed by one-way ANOVA (Statistix 3.1, Analytical Software, St. Paul, MN, USA) to test the effects of the dietary treatments. When a significant treatment effect was observed, a least significant difference (LSD) test was used to compare means. Treatment effects were considered at $P < 0.05$ level of significance. Broken line model (Robbins et al. 1979) was used to evaluate the optimum dietary level of black soldier fly larval meal as fish meal replacer in barramundi.

Results

Nutrient profile of protein ingredients

Table 2 shows the proximate composition of FM, soybean meal and BSFL meal used as the major protein ingredients in this study. The crude protein content was found to be highest for FM followed by soybean meal and BSFL meal. On the other hand, crude lipid was recorded to be highest for BSFL meal followed by FM and soybean meal. Whereas, soybean meal exhibited higher fiber content compared to FM and BSFL meal. The amino acid analysis of FM and BSFL meal has been presented in Fig. 1.

Table 2 Analyzed nutrient composition (% DM base) of fish meal, soybean meal and black soldier fly, *Hermetia illucens* larval (BSFL) meal used as the major protein ingredients in the experimental diets formulation

Ingredients	Crude protein	Crude lipid	Crude ash	Moisture	Fiber
Fishmeal	69.9	11.5	9.0	9.3	<0.1
Soybean meal	46.3	1.0	6.2	11.9	29.9
BSFL meal	43.5	16.7	13.9	5.9	9.5

Values are average from duplicate groups of samples

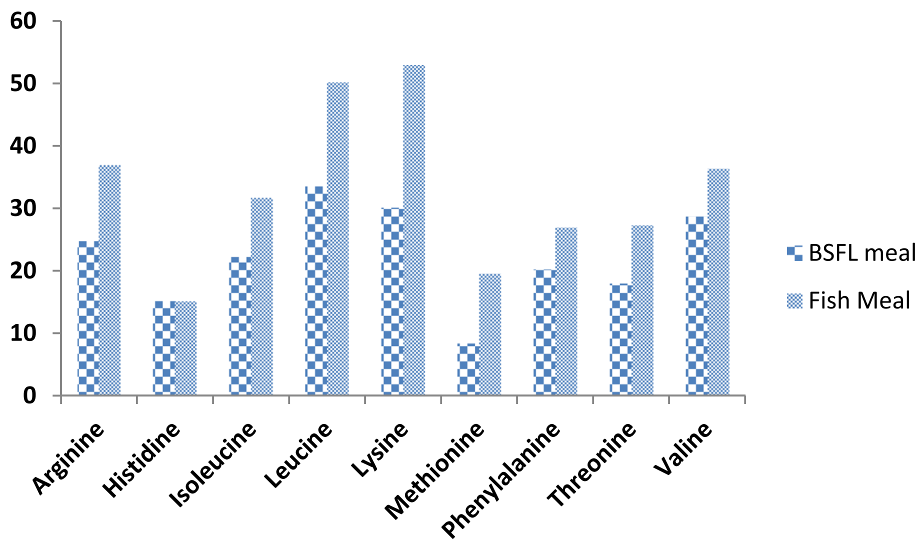

Fig. 1 Amino acid composition of black soldier fly, *Hermetia illucens* larval (BSFL) meal and fish meal. Values are average from duplicate groups of samples

Growth performances

The growth performances including weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate of barramundi-fed five experimental diets for 8 weeks have been summarized in Table 3. At the end of feeding trial, there were no significant differences in the average WG and SGR among the group of fish-fed control, $BSFL_{25}$ and $BSFL_{50}$ diets. However, WG and SGR of fish-fed $BSFL_{75}$ and $BSFL_{100}$ diets were significantly lower than that of fish-fed control diet. Whereas, data for FCR showed a significantly higher value for the group of fish-fed $BSFL_{100}$ diet than those of fish-fed all other diets. However, there was no significant difference in the FCR among the group of fish-fed control, $BSFL_{25}$, $BSFL_{50}$, and $BSFL_{75}$ diets. Average survival rate among different experimental groups numerically ranged between 80.5 and 100%. Although significant differences were recorded in the survival rate among the group of fish-fed different experimental diets, no clear trend in the effects of dietary treatments on fish survival rate could be drawn. Broken line regression analysis of the average WG indicated that BSFL meal could replace 28.4% of FM without any adverse effects on the growth performance of barramundi (Fig. 2).

Table 3 Growth performance of barramundi, *Lates calcarifer*-fed black soldier fly, *Hermetia illucens* larval (BSFL) meal as a fish meal replacer for 8 weeks

	Diets[1]				
	Control	$BSFL_{25}$	$BSFL_{50}$	$BSFL_{75}$	$BSFL_{100}$
WG (%)[2]	136.3 ± 6^{a}	119 ± 8^{ab}	110.3 ± 7^{ab}	92.3 ± 11^{b}	24.3 ± 6^{c}
SGR (%)/day[3]	2 ± 0.06^{a}	1.8 ± 0.08^{ab}	1.7 ± 0.08^{ab}	1.5 ± 0.13^{b}	0.5 ± 0.12^{c}
FCR[4]	2 ± 0.1^{b}	2.3 ± 0.1^{b}	2.4 ± 0.1^{b}	3.2 ± 0.1^{b}	11.3 ± 3.6^{a}
Survival (%)	94.4 ± 3.9^{ab}	100^{a}	94.4 ± 3.9^{ab}	80.5 ± 14.1^{b}	86.1 ± 10.3^{ab}

Means of triplicate groups of fish where values in the same row with different superscripts are significantly different ($P < 0.05$)

[1]Diets represent; control (without black soldier fly larval meal inclusion), BSFL25 (25%), BSFL50 (50%), BSFL75 (75%) and BSFL100 (100% fish meal replacement using black soldier fly larval meal)

[2]Weight gain (%) = [(final wt. − initial wt.)/initial wt.] × 100

[3]Specific growth rate (% day^{-1}) = [($\log_e$ final wt. − $\log_e$ initial wt.)/days] × 100

[4]Feed conversion ratio = feed given/wet weight gain

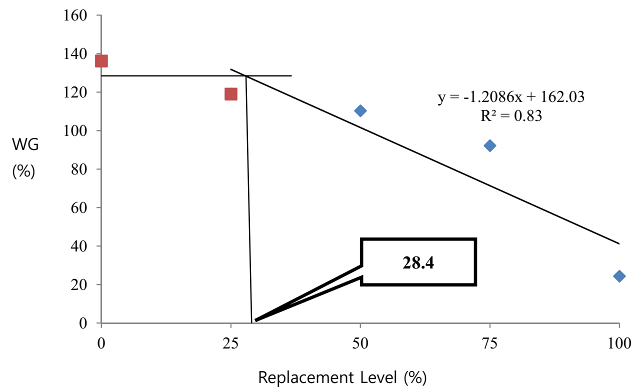

Fig. 2 Broken line regression analysis of average weight gain (WG) of barramundi, *Lates calcarifer*-fed black soldier fly, *Hermetia illucens* larval (BSFL) meal as a fish meal replacer for 8 weeks

Fish whole-body proximate composition

Whole-body proximate composition of barramundi-fed five experimental diets for 8 weeks has been shown in Table 4. Although numerical differences were recorded, crude protein and moisture content were found not to be much affected by the different dietary treatments. Whereas, fiber content was recorded highest for the group of fish-fed $BSFL_{100}$ diet.

Fish whole-body essential amino acid composition

Table 5 shows the whole body amino acid composition of barramundi-fed five different experimental diets for 8 weeks. Essential amino acids including arginine, histidine, lysine and methionine were found to be higher for the group of fish-fed $BSFL_{100}$ compared to fish-fed other experimental diets. Likewise, numerical differences were also observed for other essential amino acids, no clear trend among different dietary treatments could be drawn.

Discussion

Identifying promising alternative ingredients to minimize the inclusion level of dietary fish meal has been a much debated and extensively researched topic in aquaculture. Unfortunately, despite three decades of numerous replacement research, fish meal remains the prime protein ingredient in the majority of commercial fish feeds. Among plant protein, soybean meal is the extensively used ingredient in aquaculture diets due to its wide availability, comparatively low market price and promising nutrient profile (Akiyama et al. 1989; Gatlin et al. 2007; Trosvik et al. 2012; Cummins et al. 2013; Cummins et al. 2017). However, the price of soybean

Table 4 Whole body proximate composition (% dry weight basis) of barramundi, *Lates calcarifer*-fed black soldier fly *Hermetia illucens* larval (BSFL) meal as a fish meal replacer for 8 weeks

Diets[a]	Control	$BSFL_{25}$	$BSFL_{50}$	$BSFL_{75}$	$BSFL_{100}$
Moisture	74.3	71.9	79.2	74.4	78.0
Crude protein	62.2	63.1	55.7	69.4	66.6
Ash	15.6	15.1	17.4	19.8	19.0
Crude lipid	16.3	20.3	14.8	15.1	17.7
Fiber	6.0	6.1	4.5	3.6	8.1

Values are average from duplicate groups of samples

[a]Diets represent; control (without black soldier fly larval meal inclusion), BSFL25 (25%), BSFL50 (50%), BSFL75 (75%) and BSFL100 (100% fish meal replacement using black soldier fly larval meal)

Table 5 Whole body amino acids (ppm) composition of barramundi, *Lates calcarifer* fed black soldier fly, *Hermetia illucens* larval (BSFL) meal as a fish meal replacer for 8 weeks

Essential amino acids	Control	$BSFL_{25}$	$BSFL_{50}$	$BSFL_{75}$	$BSFL_{100}$
Arginine	6.6	6.6	6.6	6.5	9.4
Histidine	5.4	5.3	5.9	5.2	9.6
Isoleucine	1.0	1.0	1.0	1.0	0.9
Leucine	2.9	2.9	3.1	2.9	3.0
Lysine	2.8	2.8	2.8	2.7	3.2
Methionine	0.7	0.6	0.6	0.6	0.7
Phenylalanine	1.3	1.3	1.6	1.4	1.1
Threonine	2.4	2.2	2.2	2.2	2.1
Valine	0.5	0.5	0.5	0.5	0.5

Values are average from duplicate groups of samples

Diets represent; control (without black soldier fly larval meal inclusion), BSFL25 (25%), BSFL50 (50%), BSFL75 (75%) and BSFL100 (100% fish meal replacement using black soldier fly larval meal)

meal and other plant feedstuff has also increased dramatically due to their growing demand for the direct human consumption (FAO 2009). Therefore, attention could be directed towards the locally available and cheaper protein source, which may create flexibility in diet formulations and insect meal has been identified as one such ingredient (Rumpold and Schlüter 2013; Van der Spiegel et al. 2013; Van Huis 2013; Lock et al. 2015).

Black soldier fly was early been recognized as the potential candidate ingredient due to its rich nutritional profile comprised of 40% protein and 35% lipid (Bondari and Sheppard 1987). However, limited and scattered information on nutrient profile and culture techniques has restricted the use of insect larval meal in commercial fish feed formulation. Our observations for the protein content of BSFL meal closely resembled with those of previous reports, indicating 40–45% crude protein content in the larvae of black soldier fly (Yu and Chen 2009; Van Huis 2013). On the other hand, crude lipid content was found to be comparatively lower (16.7 vs. 30%) than those of values reported in these aforementioned studies. It is worthy to note that published reports on insect body composition show large differences between (Sanchez-Muros et al. 2015) and within species. It appears, the substrates used to feed the insect larvae might have profound effects on the final nutrient profile of processed larval meal. Whereas, data for amino acid comparison between fish meal and black soldier fly larval meal showed inferior but not limiting to be used in fish feed formulation, similar to the observation of Sanchez-Muros et al. (2015) with the insect, *Tenebrio molitor* meal. Overall performances for the the nutrient content (Table 2; Fig. 1) suggested, black soldier fly larvae meal as an inferior protein source than fish meal but closely similar with soybean meal.

The growth performance of barramundi, *Lates calcarifer* clearly demonstrated that larval meal of black soldier fly could be a promising alternative of fish meal in the feed of carnivorous fish species. Although WG and SGR decreased with fish meal replacement, no significant differences were recorded in these parameters among those of fish fed the BSFL-free diet and those fed-fish meal replaced with 25 and 50% of BSFL meal ($P < 0.05$). Whereas, published reports on BSFL meal as an alternative of fish meal are extremely few, but has been increasing recently (Cummins et al. 2017). Our results are in consistence with those of previous studies, suggesting an optimum fish meal replacement level of 20–50% using insect meal (Wing-Keong et al. 2001; St-Hilaire et al. 2007b; Alegbeleye et al. 2002; Cummins et al. 2017) in different aquaculture species. Similar to our observation, these aforementioned studies also reported the limitations in replacing fish meal beyond 50% of diet using insect meal. The possible reason for the reduction in fish growth performance with a corresponding increase in the dietary insect meal inclusion could be due to the substantial increase in chitin content (Sanchez-Muros et al. 2015) in addition to the deficiency in essential amino acid content (Cummins et al. 2017). Insect exoskeletons contain chitin and the b1, 4 bond in chitin has been reported to be indigestible for several fish species (Rust 2002). The ability of fish to degrade and digest chitin by the chitinase depends upon the fish species (Smith et al. 1989) and available gut bacteria. For instance, in red tilapia, *O. niloticus* × *O. hornorum* and nile tilapia, dietary fishmeal could be significantly replaced with shrimp meal, which is a rich source of chitin, without any adverse effects on the fish performance (El-Sayed 1998; Mansour 1998). Conversely, in the other species of tilapia, *O. niloticus* x *O. aureus*, the inclusion of chitin depressed growth (Shiau and Yu 1999). Our observations from the present study suggest, barramundi, *Lates calcarifer* might have limited efficiency to digest chitin thereby insect meal. Further investigation in this regard are recommended to study the chitin content in the larvae of black soldier fly and the ability of carnivorous fish species to digest chitin.

Further in the current experiment, whole-body proximate composition of fish was not much affected by the black soldier fly larvae inclusion as the fish meal replacer in the diets. Essential amino acids viz. arginine and histidine tend to be higher in the whole body of fish-fed fish meal-free diet, overall performance suggested no adverse effects of black soldier fly inclusion on the amino acid profile. Rainbow trout, *Onchrynchus mykiss* whole body showed a decreasing trend with a corresponding increase in the dietary black soldier fly inclusion (St-Hilaire et al. 2007a). Whereas, these authors found no difference in whole-body crude protein content in trout-fed different experimental diets. Likewise, in a recent study, the nutrient profile of shrimp whole body has been reported not much affected by fish meal replacement with dietary black soldier fly larval meal (Cummins et al. 2017). Our findings closely resembled with these two previous studies, suggesting negligible effects of dietary black soldier fly larval meal inclusion on the whole-body nutrient profile of aquaculture species. Overall observations from the present experiment are in agreement with those of previous reports, suggesting the potential benefit of using insect larval meal as fish meal alternative in fish feeds. Further studies are recommended to investigate the chitin content and efficiency of carnivorous fish species to digest it, with the view to further enhance the digestibility, and thereby use of insect meal in commercial fish feeds.

Conclusions

In conclusion, the current experiment opens a new avenue for the culture of insect larvae, using underutlised crop, Sesbania, *Sesbania grandiflora* as the substrate. Observations from the fish feeding trial, augment well for the use of black soldier fly larval meal as the dietary protein source in fish feeds. The maximal dietary inclusion level of protein from black soldier fly larval meal as fish meal protein replacer could be greater than 28.4 but less than 50%, characterized by the optimum growth performance, fish whole-body proximate and amino acid compositions in barramundi, *Lates calcarifer* reared in freshwater.

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