Growth performance and feed utilization of Asian seabass (*Lates calcalifer*) fed with diets containing different levels of dried housefly *(Musca domestica*) larvae meal

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Abstract

The study was conducted to examine utilization of dried housefly larvae (DFL) meal as an alternative protein source in Asian seabass diet on growth and nutrient utilization. The housefly larvae were raised in substrates composed of rice bran, discarded vegetable and fruits, molasses and water in a practical tropical environment. The obtained fly larvae were washed, dried, ground and analyzed for proximate composition. Four diets were formulated with DFL at 0, 6.78, 13.56 and 20.35 % of diet (FM at 15, 10, 5, 0%, respectively). The diets were fed to triplicate groups of each treatment twice daily for 50 days. The DFL incorporated diets up to 15% total FM replaced level had no negative effects on growth performance and nutrient utilization (p>0.05). Although statistically insignificant, the highest feed intake, final weight and specific growth rate (SGR), 25.80±1.04 (g/fish), 20.55±2.67 (g/fish) and 5.2±0.25 (%/day), respectively were in the 13.56% DFL feeding group. The total FM substituted group (DFL at 20.35% of diet) had the lowest protein efficiency ratio (PER) of 1.55±0.06 (p>0.05). Polynomial regression of percentage weight gain revealed that DFL substitution at 8% resulted in the highest growth performance which would potentially lower the cost per unit of diet for Asian seabass by 8.28%.

Keywords: Dried housefly larvae, *Lates calcarifer*, Growth, Nutrient utilization, Feed cost

1. Introduction

As the aquaculture industry has been growing rapidly with an average growth rate of 6.7% per year between 1990 and 2020 (FAO, 2022), the demand for aquafeed is adhered to the trend. In aquafeed production, protein is the most expensive component accounting for the majority of the costs. Among varieties of available protein sources, FM is considered as the most nutritious source for farmed fish due to its excellent nutritional properties, including the indispensable amino acid profile, essential fatty acids, high digestibility, and vitamins and minerals to support good growth (Olsen & Hasan, 2012; Shepherd, Monroig & Tocher, 2017). The demand for FM is noticeably increasing because of the continued stagnation or reduction of raw fish inputs from capture fisheries and the increased use of FM not only by the fast-growing aquaculture industry but also by the pig, poultry, and pet food industries which has consequently led to a rise in its price (FAO, 2022). Thus, finding alternative protein sources for aquafeed is a prime need. Several studies have successfully utilized plant-based protein sources such as soybean meal, several oil seeds and cereal gluten to replace FM in Asian seabass and various fish species (Tantikitti *et al.* 2005; Glencross *et al.* 2016; Ma *et al.* 2019, Daniel, 2018). However, substituting a large portion of FM with plant protein sources is not feasible due to the antinutritional components and non-starch polysaccharides, as well as the imbalance of amino acid and fatty acid profiles. In recent years, researchers have focused their attention on insect-based protein sources as a possible replacement for FM due to the similar nutrient composition to FM (Alfiko, Xie, Astuti, Wong, & Wang, 2022). Insects are the most diversified category of creatures and a natural food source for fish, particularly carnivorous and omnivorous fish which require relatively high protein levels in their diets (Nogales-Mérida *et al.*, 2019). Although, there is no actual protein demand for animals, but they do have a specific amino acid need (Teles, Lupatsch, & Nengas, 2011). The amount of indispensable and dispensable amino acids is a key component in determining the protein quality, which is rich in most insects (Ramos-Elorduy *et al*., 1984).

Musca domestica (family: Muscidae) is a common housefly found almost everywhere on the earth, including decomposing waste, feces, and garbage heaps (Saleh, 2020). It is among the insect species that the European Union recently approved for use in aquaculture feed production (Daniel, 2018). Housefly maggot (larvae) meal contains 50.4 % crude protein, 18.9 % lipid with good amino acid profile as compared to fish meal and commonly used soymeal meal (Alfiko, Xie, Astuti, Wong, & Wang, 2022). The potential benefits of housefly maggots as a potential protein source for fish nutrition have been previously demonstrated (Djissou, Adjahouinou, Koshio, & Fiogbe, 2016). Maggot meal incorporation has been suggested for use in diets for catfish species, but it cannot exceed 30% of diet formulation because greater inclusion levels tend to reduce growth performance (Fasakin, Balogun, & Ajayi, 2003; Okore, Ekedo, Ubiaru, & Uzodinma, 2016; Saleh, 2020). In African catfish fingerlings, maggot meal could be substitute FM at 60–70% for optimal growth and nutrient uptake (Kolawole & Ugwumba, 2018). Therefore, maggot meal might be considered a potential candidate to replace FM in fish diets.

Asian seabass (*Lates calcarifer*) is a commercially important carnivorous fish species in the Indo West Pacific region (Glencross, 2006). Aquaculture of carnivorous fish species are mostly reliant on high-energy diets that are rich in FM and fish oil (Catacutan & Coloso, 1995; Glencross, Blyth, Irvin, Bourne, & Wade, 2014; Williams *et al*., 2003). Several studies have been done replacing FM in seabass diet with alternative protein sources (Bonvini *et al*., 2018; Ma *et al*., 2019; Tantikitti, Sangpong, & Chiavareesajja, 2005). However, the potential use of dried fly larvae as a protein source in Asian seabass diets has not been investigated yet. Therefore, this study assessed the potentiality of dried fly larvae meal in Asian seabass diets in terms of growth, and nutrient utilization.

2. Materials and methods

2.1. Housefly larvae meal preparation

To obtain the fly larvae, substrates composed of rice bran, leftover vegetables and fruits, molasses, and water (3:5:0.5-1:1.5-1 by weight) were used. To attract the flies, fish heads and guts were added to lure the flies for them to lay their eggs on. The substrate ingredients were mixed in a plastic container (48 x 70 x 41 cm) and kept in shade for 4-5 days to facilitate suitable conditions for larvae production. The larvae were collected, cleaned, and dried in a hot air oven at 50 ºC for 3 days. Finally, the dried larvae were ground (Philips HR2115) and stored at -20ºC until used. The proximate composition of the ground larvae meal was determined according to the standard method of AOAC (1995). The results of the analysis of the housefly larvae meal are presented in Table 1.

2.2. Experimental diets

Four isonitrogenous (45% protein) and isolipidic (15% lipid) diets were formulated and tested using juvenile fish in a completely randomized design in triplicates. The main protein sources were FM, meat meal, and soybean meal while the lipid source was a blend of fish oil and soybean oil. The basal diet with FM at 15% of diet served as a control (0% replacement). Three FM replaced diets were produced by substituting the FM with dried housefly larvae meal at 6.78, 13.56 and 20.35 % of diet with its protein content equal to 5, 10 and 15% FM in diet, respectively. To prepare the diets, all ingredients, as shown in Table 2, were homogeneously mixed in a Hobart Mixer (Hobart LEGACY Mixer, USA) then pelleted through a single screw pelletizer that was attached to the mixer and mounted with a 3 mm sieve (pelleting retention time was 0.5 to 1 min). The throughput spaghetti-like strands were cut and the pelleted diets were dried in a hot air oven at 50 ºC for 4 hours before being stored at -4 ºC until use. The proximate composition of the diets was determined according to the standard of

AOAC (1995). Briefly, moisture content was determined by drying the diets at 105 ºC in a hot air oven until attaining a constant weight. The ash content was determined by an ash combustion method using muffle furnace at 550 ºC for 6 hours. Kjeldahl apparatus (Gerhardt, Germany) was used for crude protein determination. Crude fat was assessed by exhaustive soxhlet extraction using petroleum ether on a Soxtec System (Soxtec System HT6, FOSS TECATOR, Sweden). The amino acid profile of the diets was analyzed at Amino*Lab*, Animal Nutrition, EVONIK INDUSTRIES, Singapore. The ingredient cost and amino acid profiles of the diets are presented in Tables 2 and 3, respectively.

2.3. Asian seabass nursery and feeding

The study was performed at Kidchakan Supamattaya Aquatic Animal Health Research Center, Aquatic Science and Innovative Management Division, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Thailand. The juvenile fish were gradually acclimatized to freshwater conditions after obtaining them from the Aquaculture Technology and Innovation Research and Development Center in Songkhla, Department of Fisheries, Ministry of Agriculture and Co-operative, Thailand. They were then nursed in a fiber glass tank (1000 L) until attaining an initial body weight of 1.52±0.01 g. The fish were randomly assigned to 12 aquaria (100 L, 60 cm×40 cm×50 cm) at stocking rate of 12 fish/aquarium. Each aquarium was continuously aerated using an air blower. The feces of each aquarium were siphoned, and 80% of the water was changed daily with ground water, after treating it overnight with chlorine. The water quality indicators were carefully monitored. Fish were fed in triplicate groups with the experimental diets twice daily to apparent satiation at 8:00 and 17:00 for 50 days. Daily feed consumption was recorded for further growth performance estimation.

2.4. Growth performance and feed efficiency assessment

At termination of the feeding trial, the fish were starved for two meals before being individually weighed the next day. The growth performance indicators including final weight, weight gain, specific growth rate (SGR), average daily gain (ADG) and survival rate were assessed. To examine the effectiveness of the diets, feed intake, feed conversion ratio (FCR), feed conversion efficiency (FCE) and protein efficiency ratio (PER) were calculated as follows.

Weight gain (g/fish) = final weight (g/fish) –initial weight (g/fish)

Specific growth rate (SGR, % /day) = (lnW2-lnW1/ T2-T1) ×100; W1 = Initial weight, W2 = Final weight, T2-T1 = Cultured period (days)

Average daily gain = weight gain (g/fish) ∕ cultured period (days)

Survival rate (%) = 100× (final fish number ∕ initial fish number)

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

Feed conversion efficiency (%, FCE) = 100 × [weight gain (g/fish) ∕ feed intake (g/fish)]

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

2.5. Statistical analysis

One way ANOVA was employed to analyze all the data collected after testing for normality and homogeneity of variance by the Shapiro–Wilk W test and Levene's test, respectively, using statistical package SPSS 22 for windows. The most suitable FM substitution level for growth, using percentage weight gain, was determined using polynomial regression analysis. The Tukey's HSD test were performed to determine the differences between the treatments at 95% confidence level (p<0.05).

3. Results

3.1. Diet acceptance

The results showed that DFL substituted for FM at different levels did not affect the acceptance of the diets ($p>0.05$). Although statistically insignificant, the fish fed with the control diet and 13.56 % DFL in the diet had the lowest (23.79±3.10 g) and highest (25.80±1.04 g) feed intake, respectively (Table 4).

3.2. Survival and growth responses

The findings of the feeding trial revealed that none of FM replacement levels affected the survival rate, final weight, weight gain, SGR, and ADG of Asian seabass ($p>0.05$) (Table 4). Despite being statistically insignificant, the results showed that the growth performance measures were numerically higher when DFL was incorporated in the diets. The fish fed the diet with DFL at the substituting for dietary FM level of 10% had the highest growth performance, while those of the control group demonstrated the lowest growth. The quadratic correlation between the % weight gain (Y) and the % of FM replacement by dried fly larvae (X) were $Y = -1.5794x^2 + 24.786x + 624.13$ ($R^2=0.9993$). The polynomial regression analysis showed that the best FM substitution level for the % weight gain was 8% of FM which was equivalent to 10.85% of the diet (Figure 1).

3.3. Feed efficiency

The feed efficiency parameters including FCR, FCE and PER are presented in Table 4. The results indicate that DFL substitution in place of FM up to 15% had no significant effect on FCR, FCE and PER ($p>0.05$). Although the results were insignificant, numerically lower FCR and higher FCE and PER were observed in fish that were fed with the 5% FM replacement diet.

4. Discussion

This study examined the feasibility of using DFL in the Asian seabass diets for growth and nutrient utilization. After 50 days of the feeding trial, the survival rate (96.67±5.77

to 100.00) and feed intake suggest that fish accepted this alternative protein source made from housefly larvae very well and it had no detrimental effect on fish health. The growth and nutrient utilization indices of the four dietary groups show that DFL can completely replace FM which accounted for 20.35% of the diet. The insignificant growth response indicates that DFL met the essential amino acid requirement for good growth, as the amino acid profile of the housefly larvae meal is as good as those of FM. Despite increased levels of DFL to total FM replaced level showing a slight growth reduction tendency of the fish, the difference was insignificant. The polynomial regression curve of % weight gain suggests that 8% of FM with DFL (10.85% of the diet) can provide the best response, which will reduce the feed production cost for juvenile Asian seabass by 8.28%. The complete substitution of FM by DFL has previously been reported to decrease the growth performance in carnivorous species such as *Clarias gariepinus and Heterobranchus longifilis* (Alfiko, Xie, Astuti, Wong, & Wang, 2022). Saleh (2020) reported that a combination of 50% fresh housefly maggot and 50% formulated diet demonstrated positive growth performance in African catfish, *C. gariepinus* as compared with the fish fed either 100% formulated diet or 100% fresh housefly maggot. On the contrary, Arong and Eyo (2017) found that *C. gariepinus* fed 100% commercial feed had better growth and FCR in comparison with a 100% maggot meal or a combination of 50% commercial and 50% maggot meal diets. In tilapia, *Oreochromis niloticus,* the best growth performance and survival rate were observed at 34% maggot meal diet when maggot meal replaced FM in the diet at rates ranging from 15% to 68 (Ebenso & Udo, 2004). The growth reduction trend observed in present study, when the housefly larvae meal was included at the highest level (20.35 % of diet) might be due to a higher chitin content, that may have lowered digestibility of protein and other nutrients that support growth. Eggink, Pedersen, Lund & Dalsgaard (2022) investigated nutrient digestibility in Nile tilapia and rainbow trout fed different black soldier fly larvae meal (BSFLM) size fractions (fine, medium and course fractions). They found that the test diets containing the course-fraction BSFLM at 25% of diet lowered apparent digestibility of dry matter, crude protein, nitrogen-free extract and chitin in both fish species, as compared with other treatment groups. A similar phenomenon could explain the slightly decreased growth in our study. Further study on nutrient digestibility is therefore needed so high levels of DFL inclusion levels will facilitate and promote efficient utilization of this alternative protein source.

Generally, essential amino acid deficient diets increase the nitrogenous catabolism in aquatic animals, which pollutes the water and consequently reduces the protein retention that causes slow growth in animals. The amino acid profiles and the digestibility efficiency determine the biological value of different protein sources (Masumoto, Ruchimat, Ito, Hosokawa, & Shimeno, 1996). Furthermore, the nutritional digestibility is linked to FCR and PER (Jabir, Razak, & Vikineswary, 2012). Methionine and lysine are the most important limiting essential amino acids when unconventional protein sources are considered for use in fish diets (Djissou, Adjahouinou, Koshio, & Fiogbe, 2016). Lysine is a growth-related amino acid (Li, Mai, Trushenski, & Wu, 2009) which antagonistically interacts with arginine by enhancing the arginase activity (Cabral *et al.*, 2013). According to Millamena (1994), Asian seabass requires 2.24% methionine and cystine (0.7%), 3.8% arginine, and 4.5% lysine for proper physiological activities. All experimental diets in this study had high protein quality since they contained all the essential amino acids, particularly methionine and lysine in amounts greater than the requirements. The growth performance and nutrient utilization obtained in the fish fed the FM substituted diets indicate that the DFL, as an alternative protein source, was well utilized and satisfied the dietary essential amino acid requirements for rearing juvenile Asian seabass and conversion to muscle protein.

In conclusion, DFL can effectively substitute FM up to 15% of diet (DFL at 20.35 %) in Asian seabass juvenile diets, as an alternative protein source, without affecting survival, growth performance and nutrient utilization. Based on the polynomial regression analysis, the 8% FM substitution by DFL (equal to 10.85% of the diet) is the most efficient at reducing the costs of feed ingredients (by 8.28%) for Asian seabass diet, in so far as it performed the best in terms of growth response.

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Table 1. Proximate composition of the dried housefly larvae used in this study (% as-fed basis)

Proximate composition	**% as-fed basis**
Moisture content	22.12
Crude protein	49.09
Crude lipid	22.73
Ash content	9.73

Table 2. Composition of diets (g/100g, as-fed basis) and cost estimation of the experimental diets

Ingredients (g/100 g of diet)	**Ingredients price (bath/kg)**	**Diets (% of fish meal replacement)**			
		1 (0%)	**2 (5%)**	**3 (10%)**	**4 (15%)**
Fish meal (protein 66.55%)	40	15.00	10.00	5.00	0.00
Dried housefly larvae (protein 49.09%)	10	0	6.78	13.56	20.35
Meat meal (protein 61.31%)	25	45.78	45.78	45.78	45.78
Soybean meal (protein 46.0%)	25	10.00	10.00	10.00	10.00
Wheat flour	20	16.00	16.00	16.00	16.00
Ground rice husk	0	3.01	2.15	2.29	0.42
Soybean oil:Fish oil (1:1)	90	3.48	2.60	1.72	0.84
Vitamin and mineral premix[1]	300	4.60	4.60	4.60	4.60
CMC[2]	30	2.00	2.00	2.00	2.00
DL-methionine	225	0.13	0.09	0.05	0.01
Choline chloride[3]	109	0.51	0.51	0.51	0.51
		Cost estimation for 1 kg diet[4] (Baht)			
Fish meal (protein 66.55%)		6.00	4.00	2.00	0.00
Dried housefly larvae (protein 49.09%)		0.00	0.68	1.36	2.04
Meat meal (protein 61.31%)		11.45	11.45	11.45	11.45
Soybean meal (protein 46.0%)		2.50	2.50	2.50	2.50
Wheat flour		3.20	3.20	3.20	3.20
Ground rice husk		0.00	0.00	0.00	0.00
Soybean oil:Fish oil (1:1)		3.13	2.34	1.55	0.76
Vitamin and mineral premix[1]		13.80	13.80	13.80	13.80
CMC[2]		0.60	0.60	0.60	0.60
DL-methionine		0.29	0.20	0.11	0.02
Choline chloride[3]		0.56	0.56	0.56	0.56
Price of the diet (bath/kg)		41.53	39.32	37.12	34.91
Reduced feed cost (%)		0.00	5.32	10.63	15.93

[1]Obtained from Thai Union Group PCL, Thailand
[2]Carboxy methyl cellulose
[3]Choline chloride 5.11 g/kg diet
[4]Based on the ingredient price obtained from local suppliers (1 USD=34.18 bath)

Table 3. Proximate composition (%, as-fed basis) and amino acid profile (% in crude protein) of the experimental diets

	Diets (% of fish meal replacement by dried housefly larvae)			
	1 (0%)	**2 (5%)**	**3 (10%)**	**4 (15%)**
Proximate composition (% as-fed basis)				
Crude protein	43.20	44.21	43.99	43.89
Crude lipid	16.08	16.14	15.03	15.04
Ash	17.25	17.33	16.57	15.32
Moisture	8.55	8.93	9.29	14.89
Gross energy (kcal/g)	478.60	479.13	476.40	476.09
Amino acid (% in crude protein)				
Arginine	6.58	6.27	6.07	5.88
Histidine	2.19	2.20	2.22	2.29
Isoleucine	3.38	3.38	3.42	3.44
Leucine	6.47	6.35	6.34	6.33
Lysine	6.08	6.04	6.09	6.13
Methionine	1.99	1.88	1.81	1.73
Methionine+cystine	2.93	2.79	2.73	2.65
Phenylalanine	4.10	4.06	4.13	4.08
Threonine	3.50	3.43	3.41	3.39
Valine	4.34	4.38	4.43	4.50
Alanine	6.51	6.37	6.38	6.37
Aspartic acid	8.22	8.09	8.10	8.07
Cystine	0.94	0.91	0.92	0.92
Glutamic acid	14.01	13.73	13.69	13.65
Glycine	9.88	9.32	8.94	8.58
Proline	6.76	6.55	6.49	6.34
Serine	3.87	3.74	3.72	3.73

Table 4 Growth performance and nutrient utilization of Asian seabass fed diets containing four levels of dried housefly larvae to substitute fish meal protein for 50 days

Parameters	**Diets (% of fish meal replacement by maggot meal)**			
	1 (0%)	**2 (5%)**	**3 (10%)**	**4 (15%)**
Initial weight (g/fish)	1.52±0.01	1.52±0.01	1.52±0.01	1.52±0.01
Final weight (g/fish)	18.26±2.76	20.34±1.47	20.55±2.67	18.65±1.37
Weight gain (g/fish)	16.74±2.77	18.82±1.46	19.03±2.67	17.13±1.37
SGR[1] (%/day)	4.95±0.31	5.18±0.15	5.20±0.25	5.01±0.15
ADG[2] (g/fish/day)	0.33±0.06	0.38±0.03	0.38±0.05	0.34±0.03
Survival rate (%)	100.00±0.00	96.67±5.77	96.67±5.77	100.00±0.00
Feed intake (g/fish)	23.79±3.10	25.36±2.01	25.80±1.04	25.18±1.68
FCR[3]	1.43±0.13	1.36±0.20	1.37±0.14	1.47±0.07
FCE[4] (%)	70.27±6.11	74.79±11.66	73.60±7.75	68.04±3.35
PER[5]	1.63±0.12	1.69±0.22	1.67±0.14	1.55±0.06

Values are mean ± SD, where n=3. Means of main effects in the same column without superscripts are insignificant (*p* >0.05).

[1]Specific growth rate (SGR, % /day) = (lnW2-lnW1/ T2-T1) ×100; W1 = Initial weight, W2 = Final weight, T2-T1 = Cultured period (days)

[2]Average daily gain (ADG) = weight gain (g/fish) ⁄ cultured period (days)

[3]Feed conversion ratio (FCR) = feed intake (g/fish) ⁄ weight gain (g/fish)

[4]Feed conversion efficiency (%, FCE) = 100 × [weight gain (g/fish) ⁄ feed intake (g/fish)]

[5]Protein efficiency ratio (PER) = weight gain (g/fish) /total protein intake (g/fish)

Figure

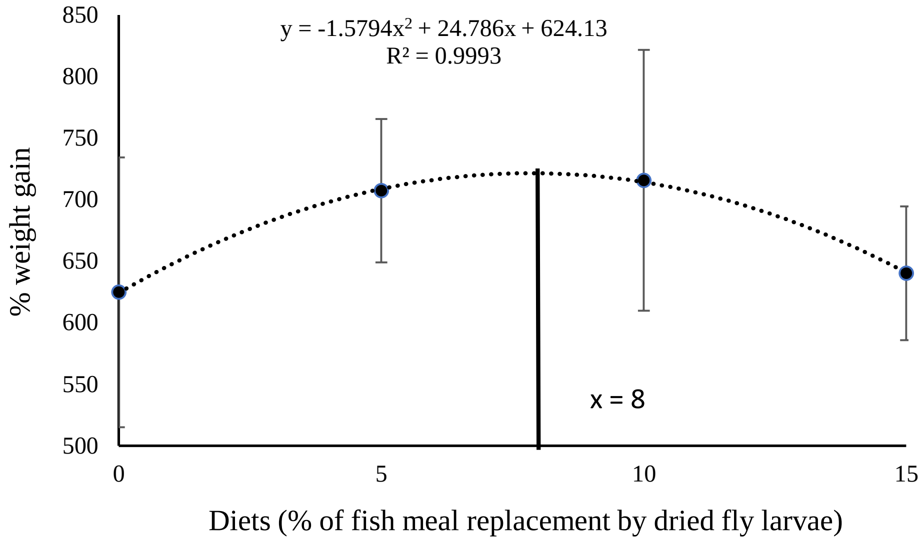

Figure 1. The optimum level of dried housefly larvae meal substituting for fish meal in Asian seabass diet based on polynomial regression analysis of weight gain (%)