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Preliminary evaluation of Superworm *(Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*

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Data availability statement (DAS)

The authors confirm that the data supporting the findings of this study are available within the article; also raw data were generated at the Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Ladkrabang, Bangkok 10520, Thailand from the laboratory of the lead author. Derived data supporting the findings of this study are available from the corresponding author elharoun@gmail.com upon reasonable request.

Abstract

A 12-week feeding trial with Asian sea bass *L. calcarifer* juveniles was conducted to evaluate the response of feeding defatted Superworm (*Zophobas morio*) larval meal on growth performance, feed utilization, apparent digestibility coefficient of nutrients and hemato-biochemical status for Asian sea bass. Five isonitrogenous (450 g/kg) and isolipidic (125 g/kg) experimental diets were formulated. A basal diet that contained no insect meal from defatted Superworm (DFSM) served as the control. Four diets were formulated where DFSM was included at 30 g/kg; 11.11% (DFSM-3), 60 g/kg; 22.22% (DFSM-6), 90 g/kg; 33.33% (DFSM-9) and 120 g/kg; 44.44% (DFSM-12), respectively on a dry matter basis. All essential amino acids (EAA) except methionine, phenylalanine and threonine in DFSM compared favorably with fishmeal (FM) and arginine, histidine, isoleucine, leucine, tryptophan and valine were higher than that of FM. No significant ($P > 0.05$) differences in weight gain, specific growth rate, and feed conversion ratio, hepato-somatic and viscera-somatic index were apparent among fish fed different levels of DFSM. Furthermore, no significant ($P < 0.05$) differences were found for apparent digestibility of dry matter (DM) and crude fat in response to dietary DFSM with different levels. A significant decreased linear relationship in the ADC of protein ($P = 0.042$) was noted as the level of DFSM incorporation increased in the experimental diet. No significant differences in hematocrit, MCHC and creatinine values were found among the experimental diets. However, a significant quadratic increase in hematocrit ($P = 0.039$) was detected as the level of DFSM increased in the diet. Serum cholesterol, alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT), glutamic oxaloacetate transaminase (GOT) were linearally reduced with the increasing of dietary DFSM (linear, $P = 0.043$; $P= 0.023$; $P = 0.018$; $P = 0.028$). The findings of the present study indicated that growth, feed efficiency and hemato-biochemical indices were not adversely affected by partial substitution of FM up to 120 g/kg dietary inclusion of Superworm meal. We discuss this in the context of Aquafeed applications for marine fish production in Asia for more sustainable production using novel protein ingredients and wider implications.

Key words: Asian sea bass, fishmeal substitution, Superworm meal, growth & feed utilization, Hematological health indicators

1. Introduction

Asian sea bass *Lates calcarifer* is an important fish species of high economic value and one of the most popular brackish-water fish cultivated in Thailand since the early 1980's (Sorphea et al., 2019). As it is a carnivorous finfish, the dietary requirements of protein are high and ranging from 450 to 550 g/kg (Glencross, 2006). The most common ingredients in Asian Sea bass commercial feeds in Thailand are usually marine originated, including fishmeal (FM) and fish oil (FO). Since, aquaculture production has been increasing every year, while annual global FM and FO production have remained constant (IFFO, 2016) there has been increasing pressure on the exploitation of marine ingredients leading to much concern to their long term sustainability. Moreover, the global demand for aquafeed and other feed ingredients has been increasing and expected to rise further due to the expanding human population and rising demand for seafood (FAO, 2018). The global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030 (Cottrell et al., 2020). Therefore, the greatest challenge for the aquaculture industry is to find eco-friendly and risk-free protein concentrates for utilization in formulated fish feeds (Glencross et al., 2019; El-Husseiny et al., 2017; 2018; Bowyer et al., 2020; Kok et al., 2020; El-Nokrashy et al 2021; Hassan et al 2021a,b). Recently, protein ingredients of terrestrial animal origin, such as insect meals, have been advocated as viable protein sources, which could potentially replace FM and plant sources such as soya bean meal in aquafeeds either partially or totally (Goda et al 2020; Hassaan et al., 2018; Davies et al 2020; Hassaan et al., 2019). The feasibility of insect meal an alternative to terrestrial plant and animal protein sources in aquaculture and animal feeds have been reported by a number of authors recently (Smetana et al., 2019; Fisher et al., 2020). One of the most promising insect species that is able to replace dietary FM is the Super worm (Zophobas morio) meal (Abd Rahman et al 2012). It can efficiently convert low-value agricultural by-products and wastes into edible proteins and fat sources

for feeding of livestock and fish (Spranghers et al., 2017). Insect meals are rich in essential amino acids particularly lysine, methionine, and leucine, and do not have any Anti-Nutritional Factors (ANF's) compared to plant by-products (Spranghers et al., 2017). More recently, Adeoyo et al. (2020) working with Afican catfish (*Clarias gariepinus*) and Weththasinghe et al. (2020) with Atlantic salmon (*Salmo salar*) have reported successful use of BSF in these fish species, respectively.

Thailand is one of the few countries of the world having developed a viable insect rearing sector to use for human food (Preteseille et al., 2018). Insect farming plays an important role in Thailand, where 20,000 farms produce around 7500 tonnes per year (Hanboonsong et al., 2013). Regarding the needs of the feed industry, insect meal such as yellow mealworm (*Tenebrio molitor*) constitutes the most relevant species used for farmed animal feed. Mealworms (*T. molitor*) are ideal candidates to cultivate in both home and factory scale since their rearing conditions have been extensively studied. Partial substitution of FM by various mealworm meals has been successfully tested in different fish species; rainbow trout (*Oncorhunchus mykiss*) (Belforti et al., 2015), African catfish (C. gariepinus) (Ng et al., 2001), (de Haro et al., 2011) and European Sea bass (*Dicentrarchus labrax*) (Gasco et al., 2016).

Superworm, *Zophobas morio* is an insect belonging to the order *Coleoptera*, known as the larva of the Darkling beetle. Superworm larvae are used to feed ornamental and exotic animals, such as lizards, frogs, birds, koi fish and other insectivorous animals (Jabir et al., 2012a). There are very few studies which have tested *Z. morio* in the feed of fish and in particular juvenile Asian sea bass (*L. calcarifer*). Furthermore, the application of a defatting process could enhance the protein content and improve availability (Choi et al., 2017). Therefore, this study was undertaken to determine the effects of a partial dietary inclusion of a defatted Superworm meal DFSM for Asian Sea bass (*L. calcarifer*).

This was a preliminary feasibility study testing this novel protein source due to its current limited availability and cost.

126 We examined the Superworm insect larval meal as an alternative to FM as a protein source,
127 and its influence on the growth performance and feed utilization efficiency, apparent digestibility
128 coefficient of nutrients, and hemato-biochemical status in a 12-week feeding trial with juvenile Asian
129 sea bass.

130 **2. Materials and methods**

131 *2.1 Preparing Superworm larval meal*

132 Larvae of the Superworm (*Z. morio*) were raised on a feedstock composed of feed grade
133 materials by the Meat and Protein Innovation Technology Center (MPITC), Faculty of Agricultural
134 Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok Thailand. The
135 Superworm (45 days old) was ground; extracted using the solvent extraction by ethanol (2:1 w/v) and
136 the liquid part was decanted. The remaining Superworm paste was then dried at 65ºC overnight and
137 ground into a fine powder and stored at 20º C until used in the feeding trial. The chemical composition
138 of the full fat Superworm and defatted worm meal together with fishmeal is shown in Table 1.

139 2.2. *Determination of amino acid profiles of experimental dietary ingredients*

140 The amino acid compositions of insect meals from Superworm was determined using an
141 automated amino acid analyzer (LAA-A; Basing View, Basingstoke, Hampshire RG21 4RG, UK)
142 after hydrolyzing the samples with 6 M HCl at 110°C for 24 h (Bassler and Buchholz, 1993). Sulphur-
143 containing amino acids were oxidized separately using 4M performic acid (CH_2O_3) before the acid
144 hydrolysis stage. These are shown in Table 2 and compared to fishmeal expressed both as g kg^{-1} (DM
145 ingredient) and g kg^{-1} of crude protein.

146 *2.3. Diet preparation and physical properties of pellets*

147 Five isonitrogenous (450 g/kg^{-}) and isolipidic (125 g/k g) of experimental diets were
148 formulated to meet the standard specifications of the commercial Asian sea bass feed by the
149 Department of Fisheries, Thailand, and nutrient requirement recommendations by Glencross (2006)
150 for this species as shown in Table 3. Chemical composition of fish and diet samples were determined

151 according to the procedures of the AOAC (2000). The basal diet contained no insect meal from
152 defatted Superworm (DFSM). Four dietary treatments were designed to include DFSM at levels of
153 30 g/kg (DFSM-3), 60 g/kg (DFSM-6), 90 g/kg (DFSM-9) and 120 g/kg (DFSM-12) at the expense
154 of fishmeal (tuna fishmeal byproduct) on a dry matter basis. The formulation of the experimental
155 diets and nutrient analysis is presented in Table 3. All the diets were produced at the Aquafeed Mill
156 factory of the center of Meat and Protein Innovation Technology (MPIT), Faculty of Agricultural
157 Technology, KMITL. Bangkok, Thailand. Briefly, all the dry ingredients were ground to the particle
158 size of ~500 micron (μm) using a hammer mill with an air-assist system. Mash feeds were automatic
159 transferred into a horizontal mixer (with added water) and vitamins, minerals, and chromic oxide
160 mixtures were then hand-added and mixed using a horizontal single-shaft ribbon mixer. The diets
161 were extruded using specific extruding conditions (3.0 mm of die hole to obtain a 4.5 millimeters of
162 diet) employing a single-screw extruder. The wet extrudates were air dried at 190 ºC using a rotary
163 drum dryer for about 10 minutes. The pellets were then coated by all liquid ingredients (fish oil and
164 fish solubles) using a sprayed drum coater. All the diets were transferred to an air cooler, packaged
165 into respective sealed bags and kept at room temperature until use.
166 The effect of insect meal inclusion from Superworm (defatted) on the pellet quality was also
167 evaluated by measuring bulk density. The bulk density was measured for each diet after drying
168 according to the method described in Sørensen (2012)
169 *2.2 Experimental fish and feeding*
170 Asian sea bass, *L calcarifer* juveniles with no clinical signs of disease were obtained from a
171 local commercial hatchery in Petchaburi province of Thailand. The fish were acclimated in the
172 freshwater flow-thru system in the wet laboratory of the Department of Animal Production
173 Technology and Fisheries, KMITL for 15-day. After acclimation, fish were randomly distributed into
174 15 indoor rectangular aquaria (24 x 30 x 38 inch) with three replicate aquaria assigned for each of the

five dietary treatments (DSM-0, DFSM-3, DFSM-6, DFSM-9 and DFSM-12) according to a completely randomized design (CRD).

Twenty-five fish with an average body weight (12.52± 0.52 g) $fish^{-1}$ were randomly sampled and allocated to each aquarium. All the aquaria were connected to a freshwater semi-recirculating system with continuous oxygen supply. Fish were hand fed until the visual satiation 2 times daily (08:00 and 16:00 h) and 6 days per week over the 12-week feeding trial. The rearing conditions were as follow: water temperature (25.5 ± 0.5 °C), dissolved oxygen (6.59 ± 0.20 mg L-1) using a portable DO meter, and pH (8.1 ± 0.4), total-ammonia nitrogen (0.005 ± 0.002 g L^{-1}) was measured using a digital multimeter (Crison, model MM41, Spain) and a photoperiod regime (12:12 h, light: dark) was used throughout the trial period.

2.3 Fecal collection for digestibility study

The apparent digestibility coefficients (ADCs) of different experimental diets measured using chromic oxide (Cr_2O_3) as an external marker at a level of 5 g kg^{-1} diet. After one month of feeding, the fecal samples were collected by hand siphoning from each aquarium every morning before the start of feeding. The feces were collected on filter paper for drying according to the protocol of Prachom et al. (2013). The collected feces were dried in an oven at 50ºC for about 24 hours and stored at -20 ºC (Godoy et al, 2016; De Silva et al, 1990) until further chemical analysis.

2.4 Sampling procedure

At the beginning of the experiment, a pooled sample of 15 fish juveniles was sampled for the initial whole-body composition. Sampling was conducted every 2 weeks to measure growth parameters. Fish were starved for 24 h before weighing and sampling. At the end of a 12-week feeding trial, five fish were randomly sampled from each aquarium for the final whole-body composition. Five fish from each aquarium were used to withdraw blood samples. Blood was drawn by by using a heparinized syringe (2000 unit mL^{-1}) near the caudal vein and transferred into a 5-mL Eppendorf tube. Whole blood samples were used for determination of hematocrit, hemoglobin and mean

corpuscular hemoglobin concentration. Blood samples were collected, then centrifuged at 3000 g for 15 minutes at 4 °C and serum was collected. Serum samples were kept at -35 °C and used for determination of serum enzymes. Fish were then dissected for liver, muscle, visceral organs (including heart and kidney) and abdominal fat. The weight of each part was recorded and used for determining biological indices.

2.5. Growth performance and feed utilization parameters

The final body weight (FBW) was assessed by dividing the total fish weight (weighing each individual separately) in each tank by the number of fish. The weight gain (WG), specific growth rate (SGR), survival (%), feed conversion ratio (FCR) and protein retention efficiency (PRE) were determined using the following equations:

WG (g $fish^{-1}$) = Final weight – Initial weight

SGR (%day^{-1}) = 100 × (In Final weight- In Initial weight) / days

Survival (%) = 100 × (Final number of fish/Initial number of fish).

FCR = Feed intake (g) / Weight gain (g).

PRE (%) = Protein gain (g) / Protein intake (g) × 100

2.6. Diets and whole-body proximate chemical composition and digestibility estimation

Analysis methods of the AOAC (2000) were followed to determine the diet and the chemical compositions of whole-body fish samples. Dry matter (DM) was assessed after drying the samples at 105 °C for 24 h then Ash was evaluated following incineration at 550 °C for 12 h.

Crude Protein (CP) was estimated by Dumas method, with N% × 6.25 (using Carbon/Nitrogen Determinator, Leco CN 628 Model, U.S.A.). Crude Fat (CF) was determined by automated lipid extraction machine (Buchi Scientifica, E-816 Model, Switzerland). Crude Fiber (CF) in diets was estimated after digestion with 5% sulfuric acid and 5% sodium hydroxide for 15 min; the residues were then dried and ashed. Amino acid in samples was determined by amino acids analyzer using

amino acid analyzer (Hitachi L-8900 Model, Japan). An acid digestion method was used to analyze chromic oxide content in feces and diets (AOAC, 1995; Davies and Gouveia, 2006).

The absorbance was measured using a spectrophotometer set at a wave length of 350 nm. Apparent Digestibility Coefficient (ADC) of protein, fat and dry matter were obtained using the following equations:

Chromic oxide content (%) = W_{Cr}/W_{sample} x 100

Where, W_{Cr} = weight of chromic oxide in the sample (mg) and W_{sample} = Weight of the sample (mg)

W_{Cr} = (Y – 0.0032)/0.2089

Where, Y = Absorbance (at 350 nm)

ADC of nutrient (%) =

100 – 100 x (Chromic oxide $_{diet}$ x Nutrient $_{faeces}$)/ Chromic oxide $_{diet}$ x Nutrient $_{faeces}$)

ADC of dry matter (%) = 100 – Chromic oxide $_{diet}$/Chromic oxide $_{faeces}$ x 100)

2.6. *Haemotogical and biochemical parameters*

The hematological parameters are expressed in international units (SI). The total red and white blood cell counts (RBC; 106 mm-3 and WBC; 103 mm-3, respectively) were obtained by using a standard Neubauer-hemocytometer chamber using Shaw's solution as diluting fluid (Stoskopf, 1993). Hemoglobin (Hb; g dL-1) was assayed colorimetrically using commercial kits according to the cyan-methemoglobin procedure (Makarem, 1974; based on Drabkin's reagent). Hematocrit (Hct) were determined by using microhematocrit-heparinized capillary tubes and a microhematocrit centrifuge (10000 g for 5 min) Levels of serum ALP, GPT and GOT (glutamic oxaloacetic transaminase) were assayed according to the methodology described by Reitman & Frankel (1957) and further described by Hassaan et al. (2019) and modified to perform on a Biosystems BA400 (Spain) automated analyzer at the Veterinary Central Laboratory, Lorong 4 Toa Payoh, Bangkok, Thailand.

2.7 *Statistical analysis*

Linear and quadratic regression analysis were used to evaluate the relationship between level of DFSM and measured indices; growth performance, somatic index, digestibility, hematology, serum biochemical analysis and proximate composition. All data were analyzed by using the software SAS, version 6.03 (Statistical Analysis System 1996).

3. Results

3.1 Amino acid of defatted Superworm

The content of essential amino acids (EAA) in DFSM compared to fishmeal (FM) showed that arginine, histidine, isoleucine, leucine, tryptophan and valine were higher than that of FM, whereas, the content of cysteine, methionine, phenylalanine and threonine was lower than FM as observed in Table 2**.**

3.2. Physical properties of pellets

Physical properties of pellets such as bulk density and water stability after extrusion of the experimental diets were not affected with increasing level of DFSM (Table 4).

. 3.3. Growth performance of Asian sea bass

Fish in all treatments appeared normal and did not exhibit any clinical signs of disease or abnormalities during the entire feeding period. The growth performance and feed utilization efficiency of juvenile Asian Sea bass fed diets with different inclusion levels of Superworm, (defatted) are presented in Table 5. No significant ($P > 0.05$) differences in Weight Gain, WG and Specific Growth Rate, SGR among fish fed different levels of DFSM were observed. Different levels of DFSM in diets had no effect on feed intake of Asian Sea bass ($P > 0.05$). Similarly, Feed Conversion Ratio, FCR were the same among fish fed different levels of DFSM, and the values ranged from 1.36-1.37 (Table 5). There were no significant ($P > 0.05$) differences noted in the morphometric indices (HIS and VSI) among fish treated with different levels of DFSM.

3.4. Apparent digestibility coefficient of experimental diets

Apparent digestibility coefficient (ADC, %) of dry matter, protein and fat in experimental diets are presented in Table 6. No significant ($P > 0.05$) differences were found for apparent digestibility of dry matter and crude fat in response of dietary DFSM with different levels. Significant decreased linear trend in ADC of protein (Figure 1; $P = 0.042$) was noted as the level of DFSM increased in the experimental diet. At the maximum desirability (0.95), the highest ADC of protein values was 92.7 for fish fed DFSM-0, while the lowest value recorded for fish fed DFSM-12.

3.4 Whole-body composition

Chemical analysis of fish fed diets with different levels of DFSM is displayed in Table 7. Increasing inclusion of DFSM level did not affect chemical composition; moisture, crude protein, lipid content and ash content of Asian sea bass at the end of the feeding trial of 12-weeks.

3.5 Haemato-biochemical profile

Data of the haemato-biochemical profile of Asian sea bass at the end of the trial are presented in Table 8. The hematocrit values varied between 11.0 and 12.6 g dL^{-1}, MCHC contents varied between 33.0 and 34.9 %, creatinine values varied between 0.39 and 0.42 among the experimental diets without significant ($P > 0.05$) effect of DFSM. A significant (quadratic) increase in hematocrit ($P = 0.039$) was detected as the level of DFSM increased in the diet. Cholesterol, GPT and GOT linearally declined with the increasing inclusion of dietary DFSM (linear, $P = 0.043$; $P = 0.018$; $P = 0.028$) from 30 to 120 g/kg in each diet group respectively. While, a quadratic ($P = 0.023$) trend was observed in the ALP activities for fish fed different levels of DFSM (Figure 2)

4. Discussion

The quality of new ingredients plays an important factor to achieve their optimum incorporation level to replace fish meal in aquafeed formulations. This is a prerequisite in meeting the stringent specifications for the industry. It should also be noted that the nutritional contribution of these ingredients may not be fully realized even if they possess high nutritional values, if they do

not exhibit good physical properties for pelleting of finished diets (Aas et al., 2011). Extruded feed is linked with numerous physical characteristics (i.e. sinking, slow sinking, floating, etc.) as well as the specific aquaculture feed management requirements of the species in question. Thus, physical properties such as bulk density which controls pellet sinking rates is desired to achieve optimized quality of extruded feed. The addition of DFSM (Defatted Superworm Meal) to an extruded diet for Asian sea bass, *Lates calcarifer* did not affect bulk density or buoyancy characteristics of pellets and feeding acceptability was good with inclusion of DFSM. This finding was in agreement with those recently expressed by Weththasinghe et al., 2020 who tested the inclusion of full fat BSF meal either as a dry meal or paste in experimental diets for Atlantic salmon, *Salmo salar* without detriment to pellet quality characteristics.

In terms of nutritional value, the essential amino acid profile, EAA of Defatted Superworm Meal DFSM is of a similar pattern (with lower relative levels) compared to a standard low temperature LT dried fishmeal (FM) giving it potential as an alternative source for FM for Asian sea bass feeds. The EAA composition of DFSM in the current study seems sufficient to meet known requirements for most marine species, with no obvious deficiency of essential amino acids when compared to European sea bass (Oliva-Teles, 2006), although levels of methionine, phenylalanine and threonine contents in DFSM were slightly lower than in FM. However, the highest substitution level of FM (120 g/kg) by DFSM did not affect the growth performance of Asian sea bass in our study (Table 5). The same finding was observed by Jabir et al. (2012b) who reported that all essential amino acid (EAA) content in insect meal of Superworm was equal with that of FM except for methionine. However the sulphur amino acid cysteine could partially satisfy the requirement of methionine with varying efficiency of substitution and sparing action (NRC, 2011). On the other hand, the protein content and essential amino acid content in Superworm meal can often show significant variation (Finke and Winn 2004; Ojewola et al., 2005). These variations may be due to many factors such as different larval/age, feeding and nutritional status, conditions of production and differing processing

methods. Chemical composition such as crude protein and lipid content in DFSM used in this study is 614 g/kg and 127 g/kg, respectively (Table 1), while crude protein in Superworm meal of the study of Jabir et al. (2012b) was somewhat lower at 50.5.3 g/kg.

Growth performance and feed utilization of juvenile Asian sea bass in the present investigation showed no significant differences among the treatment groups. These results indicated that diets containing DFSM are suitable and well digested by Asian sea bass without negative effect on growth and feed conversion as well as other important performance indicators. Furthermore, different dietary inclusion levels of DFSM had no deleterious effects on diet palatability and feed acceptance and intake of fish. The results are consistent with those of Fisher et al. (2020) who found that insect meal could partially or totally replace fishmeal without negative effects on the performance of Atlantic salmon (*Salmo salar*). Furthermore, Li et al. (2017) reported that FM was totally replaced by defatted black solder fly larvae meal in diets of Jian carp (*Cyprinus carpio*) without any appreciable effect on growth and feed utilization efficiency. The matching growth performance noticed of the DFSM group versus the FM fed sea bass diet in the present study maybe due to i) DFSM contains a high quantity of protein (~650 g/kg), lipid (100–300 g/kg) and the pattern of balanced essential amino acids of DFSM, which is fairly alike to fishmeal (Tacon and Metian, 2013), FM has become the main reference 'ideal' protein meeting the optimum essential amino acid profile for fish; ii) insect meals do not have any identifiable Anti-Nutritional Factors (ANF's) (Spranghers et al., 2017); iii) Furthermore, DFSM is also a good additional source of minerals and vitamins (Fisher et al., 2020).

No significant differences were found in the organ somatic indices i.e. HSI, VSI among treatment groups for Asian sea bass. Similarly, Zhou et al. (2018) also reported that HSI and VSI of Jian carp fish were not affected by inclusion level of defeated black solder fly larvae meal. Likewise, no significant differences in HSI, VSI were reported for Japanese sea bass (*Lateolabrax japonicus*) fed diets containing black solder fly larvae meal (Wang et al., 2019). Recently, Adeoye et al. (2020)

evaluated black soldier fly larvae for African catfish *Clarias gariepinus* in a comprehensive nutrition assessment with favorable results on growth rates, feed utilization, hematological and several specific blood biochemical indices for health. The proximate chemical composition of the whole-body of Asian sea bass showed there were no significant differences ($P \geq 05$) observed in moisture, crude protein, total lipids, and ash contents among treatments. These results are in alignment with Zhou et al. (2018) who also reported no significant differences in chemical compositions of body, muscle tissue, and hepatopancreas of carp fed diets containing Defatted Black Soldier Fly Larval Meal (DBSFLM). Also, Gasco et al. (2016) reported no differences of European sea bass whole body chemical composition fed *Tenebrio molitor* (TM) meal. On the contrary with our current study on Asian sea bass, Belforti et al. (2015) found decreases ($P \leq 0.05$) of moisture and total lipid content, and an increase ($P \leq 0.05$) of crude protein content of turbot juveniles fed Defatted Black Soldier Fly Larval Meal, DBSFLM in their experimental diets for this species.

In our study, the apparent digestibility coefficients ADC's of protein and lipid was significantly altered with high inclusion level of DFSM, and the lowest value was observed in DFSM-12 (88.7 and 95.7), respectively. The ADC of dry matter was not affected by inclusion level of DFSM. Similar results were found in ADC of dry matter, protein, and lipid for red tilapia fed different substitution levels of Superworm (Jabir et al., 2012b). While, ADC of dry matter in the present study was lower than the value of 840–890 g/kg reported by Eusebio et al. (2004). The ADC of dry matter may be affected by the type of raw material employed for rearing of insect larvae. A similar result was obtained when substituting FM with housefly (*Musca domestica*) maggot meal in the diet of *O. niloticus* fingerling (Ogunji et al., 2007; Wang et al., 2017). The lower ADC for crude protein of DFSM may be due to lower content in some essential amino acids like methionine (Table 2). In the present study, the authors showed that up to 540 g/kg replacement of FM by DFSM; ADC of protein was not affected, confirming that the Asian sea bass have a good ability to digest the DFSM protein at his level. It should be noted that acquiring digestibility data for Superworm is deemed

necessary for future more refined experimental diets based on DCP (Digestible Crude Protein) and DE (Digestible Energy) values for correct ingredient manipulation in diets. Ideally we will also require accurate digestible amino acid profiles to further optimize the formulations for essential amino acid balance.

Monitoring of hemato-biochemical parameters are widely used in feeding trials and reflect health status, and the ability of fish to adapt with different feed composition and culture conditions (Hassaan et al., 2018; Davies et al., 2019; Davies et al., 2020). Hemato-biochemical measurements are frequently used to monitor metabolism, physiological changes and fish behavior to adapt and mitigate varying environmental conditions (Abdel- Tawwab, 2020). In the present investigation, no significant differences in the standard hematological indices were detected by the effect of the dietary inclusions level of DFSM in diets of Asian sea bass (Table 8). Abdel-Tawwab et al. (2020) reported that hematological and serum biochemical; ALT (Alanine Aminotransferase) AST (Aspartate Aminotransferase), total protein, albumin and globulin of European sea bass, *Dicentrarchus labrax* were not affected by substitution of FM by Defatted Black Solder Fly Meal (DBSFLM). Determination of selected marker enzymes in the blood of Asian sea bass AST and ALT is indicative of general systemic physiological status as well as the resilience of the vascular system and hepatic function (Kumar et al., 2011). Increased activaty of serum AST and ALT in fish may reveal possible leakage of these specific enzymes across ruptured cell membranes and/or increased synthesis of enzymes by the liver (Hassaan et al., 2019). In our investigation, the inclusion of Superworm meal indicates a functional improvement in fish health as shown by linear and significant downward trends in the serum activities of AST, ALT and GOS for Asian sea bass fed incremental dietary level of worm meal. Similarly Wan et al. (2017) reported an improvement of several blood health indices for a study examining the effects of silkworm pupae meal and invertebrate meal (marine polychaete worm meal) for mirror carp (*Cyprinus carpio*). A possible explanation could be the enhanced complementation of dietary essential amino acids with elevated protein assimilation and less hepatic

lipid and glycogen accumulation (hyperlipidemia or hyperglycemia) although not tested in this study. In contrast, serum biochemical profile of Jian carp fed dietary inclusion levels of defatted black solder fly meal were not significantly affected as reported by Zhou et al. (2018) and by a regular commercially sourced BSF meal in African catfish diets (Adeoye et al., 2020). Likewise, the replacement of FM in a basal diet of Jian carp and African catfish with different levels of DBSFLM and BSFM resulted in no differences in serum of glucose, total protein, albumin, globulin, ALT, and AST (Adeoye et al., 2020).

In summation, more studies are warranted to validate the efficacy of insect larval meal of Superworm (*Z. morio*), since nearly all the contemporary scientific literature concerns the use of black solder fly larvae meal whereas many more insect derived meal sources are becoming available. Cost, uniform quality and availability of these ingredients has been a constraint and commercial feed manufacturers have limited insect meal inclusion in diets for fish within a mixture of protein alternatives in current feed formulations. Superworm could therefore effectively replace up to 500 g/kg of the fishmeal formulated diets for Asian sea bass and it should be noted we did not exceed the critical threshold level of around 120 g/kg for fishmeal diet inclusion as reported for Barramundi by Glencross et al. (2010). It will be important to evaluate higher inclusion levels to replace secondary as well as primary protein ingredients and to test these novel worm-based meals at varying stages of production from niche applications for hatchery rearing of fry to on-grower diets and towards harvest. In the latter phase we should also address the question of taste and consumer perception of Asian sea bass fed worm meal ingredients. Most studies have also focused on growth performance of freshwater fish species and our work is one of the few on Asian marine fish of commercial importance and high value. Further work is obviously warranted to evaluate even higher inclusion levels of Superworm meal in feeds extending the upper threshold by substitution of other ingredients such as plant by-products (e.g. soybean and corn) and animal by-products like poultry meat and feather meals.

Dissemination of findings to other marine species is important to S.E Asia for sustainable production in aquaculture.

Conclusion

The source of alternative protein concentrates such as terrestrial invertebrate insect meals can become a viable choice in meeting the same quality as fish meal in diets for marine fish in Thailand and beyond. Notably this study revealed that fishmeal could be partially substituted by dietary defatted Superworm meal, DFSWM protein in juvenile Asian sea bass diets using up to 120 g/kg inclusion without imparting negative effects on production performance.

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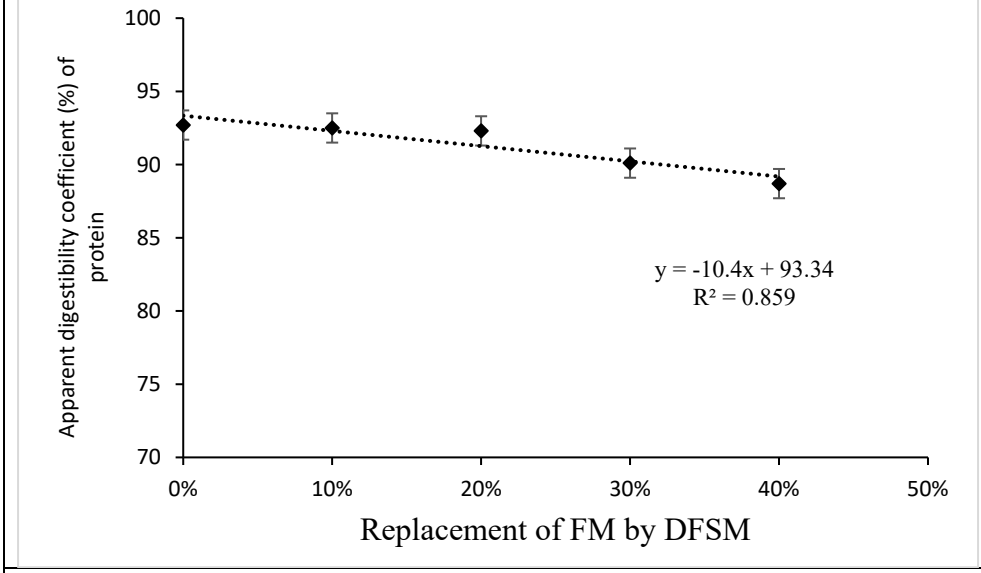

Figure 1. Apparent digestibility coefficient protein of Asian sea bass, *L calcarifer* fed the experimental diets after 12-weeks

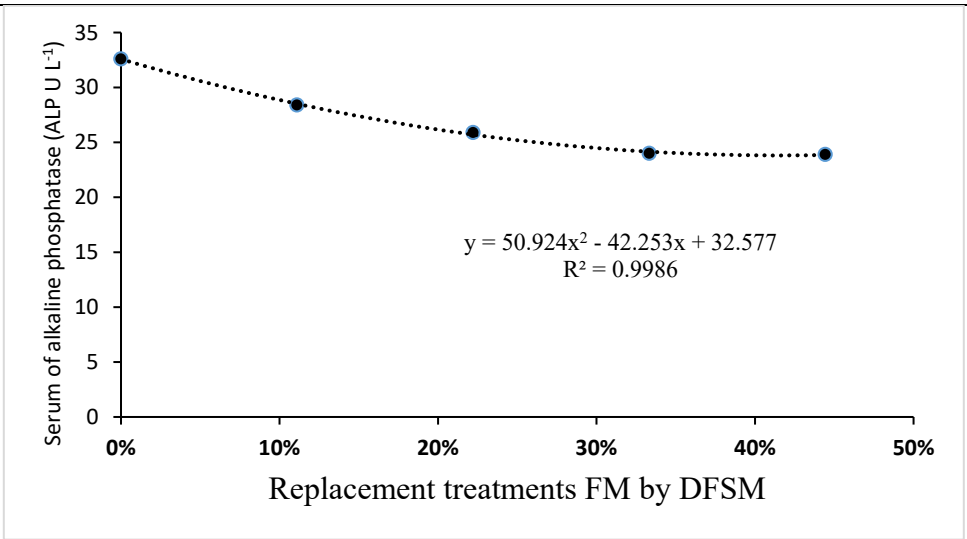

Figure 2. Activities alkaline phosphatase (ALP) of Asian sea bass, *L calcarifer* fed the experimental diets after 12-weeks

Table 1. Chemical composition (g/kg) of fishmeal grade 60 percent protein, defatted and full fat insect meal prepared from Superworm (*Zophobas morio*) on a dry matter DM basis

Chemical composition	Fishmeal	Defatted Superworm	Full fat Superworm
Protein	609	614	468
Fat	129	127	417
Ash	161	47	36
Fiber	4	40	26
Dry Matter	909	662	652
Energy MJ Kg^{-1}	20.5	21.7	24.8

Table 2. Amino acid g/kg of fishmeal grade, defatted and full fat insect meal prepared from Superworm (*Zophobas morio*) on dry matter and g/kg of crude protein in brackets

Amino acid	Fishmeal	Defatted Superworm	Full fat Superworm
Essential amino acids			
Arginine	36.8 (60.3)	15.8 (46.8)	14.4 (35.2)
Histidine	15.6 (25.6)	7.6 (22.4)	7.6 (18.6)
Isoleucine	30.6 (50.2)	9.9 (29.6)	8.9 (21.9)
Leucine	50.0 (82.0)	16.0 (47.2)	14.9 (36.6)
Lysine	51.1 (132.2)	18.2 (53.9)	17.9 (44.0)
Methionine	19.5 (31.4)	3.7(10.8)	3.7 (9.0)
Phenylalanine	26.6 (43.6)	7.7 (22.9)	8.1 (19.7)
Threonine	28.2 (46.2)	8.3 (24.5)	8.4 (20.5)
Tryptophan	7.6 (12.3)	2.6 (7.8)	2.0 (4.9)
Valine	35.1 (56.6)	12.5 (36.8)	11.2 (27.5)
Non-essential amino acids			
Cysteine	42	1.2 (3.6)	1.5 (3.7)
Tyrosine	N.A.	12.5 (37.0)	13.8 (33.9)

Table 3. Dietary formulations, proximate composition and energy content of the experimental diets (g/kg)

Ingredients (g/kg)	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12
Tuna fishmeal, byproduct	270	240	210	180	150
Superworm, defatted	0	30	60	90	120
Soybean meal, dehulled	180	180	180	180	180
Poultry by-product meal	125	125	125	125	125
Corn seed meal	40	40	40	40	40
Wheat flour	210	210	210	210	210
Wheat gluten meal	60	60	60	60	60
Soybean oil	20	20	20	20	20
Tuna fish oil	55	55	55	55	55
Fish solubles	20	20	20	20	20
Vitamin-Mineral premix [1]	15	15	15	15	15
Mono calcium phosphate	3.5	3.5	3.5	3.5	3.5
Antioxidant [2]	0.5	0.5	0.5	0.5	0.5
Chromic oxide (Cr_2O_3)	0.5	0.5	0.5	0.5	0.5
Antimicrobial agent [3]	0.5	0.5	0.5	0.5	0.5
***Proximate compositions*, g/kg DM**					
Ash	127	124	127	123	123
Protein	455	455	455	454	454
Fat	126	125	125	124	124
Fiber	22	21	22	22	22
Nitrogen free extract [4]	277	275	276	280	278

[1]Vitamin-mineral premix (unit.kg-1): A 12,000,000 IU, D3 2,200,000 IU, E 100,000 mg, K3 12,000 mg, B1 25,000 mg, B2 25,000 mg, B6 23,000 mg, B12 43 mg, Pantothenic 75,000 mg, Niacin 125,000 mg, Folic 4,000 mg, Biotin 800 mg, Copper 80 mg, Iron 150 mg, Manganese 50 mg, Zinc 120 mg and Selenium 0.3 mg)

[2]Antioxidant (Butylated hydroxyl toluene and Butylated hydroxyl anisole)

[3]Antimicrobial agent (Formic acid, Propionic acid and Benzoic acid)

[4]Nitrogen free extract (NFE) is calculated by 100 – (Protein + Fat + Fiber + Ash)

Table 4. Bulk density and water stability of experimental diets post extruded

Ingredients, %	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12
Bulk density, g L^{-1}	438.5	437.0	433.3	433.8	432.4
Water stability 1 hour, %	85.7	85.8	85.7	85.8	85.9

Table 5. Growth performance, feed utilization efficiency, hepato-somatic index (HSI) and viscera-somatic index (VSI) of Asian sea bass, *L. calcarifer* fed the experimental diets over a 12-week period

Parameters	Experimental diets					SEM	*P value*	
	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12		Linear	Quadratic
IBW[1] (g fish^{-1})	12.52	12.45	12.5	12.46	12.52	0.52	0.562	0.695
FBW[2] (g fish^{-1})	74.96	75.41	75.25	74.95	74.96	0.98	0.452	0.523
WG[3] (g fish^{-1})	62.44	62.96	62.75	62.49	62.44	0.18	0.523	0.258
SGR[4] (%, day^{-1})	1.43	1.43	1.43	1.43	1.43	0.01	0.235	0.456
FI[5] (g fish^{-1})	71.73	71.66	71.58	71.23	71.73	0.13	0.956	0.233
FCR[6]	1.15	1.14	1.14	1.14	1.15	0.04	0.132	0.423
PRE[7] (%)	32.44	32.28	32.21	32.07	32.44	0.25	0.892	0.335
HSI (%)	0.87	0.80	0.84	0.99	0.87	0.05	0.569	0.789
VSI (%)	4.78	5.05	5.17	5.71	4.78	0.42	0.878	0.689

[1]IBW = Initial body weight
[2]FBW = Final body weight
[3]WG = Weight gain
[4]SGR = Specific growth rate
[5]FI = Feed intake
[6]FCR = Feed conversion ratio
[7]PRE = Protein retention efficiency

Table 6. Apparent Digestibility Coefficients of dry matter, protein and fat in experimental diets at the end of the 12-week study

Parameters	Experimental diets					SEM	*P value*	
	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12		Linear	Quadratic
Dry matter	77.8	78.4	78.1	78.3	78.7	0.32	0.562	0.695
Protein	92.7[a]	92.5 [a]	92.3 [a]	90.1 [ab]	88.7 [c]	1.21	0.042	0.923
Fat	96.7	96.5	96.3	96.1	95.7	1.81	0.523	0.258

Table 7. Final proximate composition of whole body of Asian Sea bass, *L. calcarifer* (g/kg) fed the experimental diets (express in g/kg wet basis) (n= 3) after 12-weeks

Parameters	Experimental diets					SEM	*P value*	
	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12		Linear	Quadratic
Dry matter	27.3	27.8	28.1	27.6	28.1	1.1	0.236	0.321
Protein	179	177	177	176	173	0.20	0.879	0.231
Fat	44	44	44	47	49	0.12	0.231	0.891
Ash	14	14	13	13	13	0.01	0.698	0.238

227 **Table 8.** Hematocrit, hemoglobin and mean corpuscular hemoglobin concentration (MCHC),
228 creatinine, cholesterol, alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT),
229 glutamic oxaloacetate transaminase (GOT) of Asian sea bass, *L calcarifer* fed the experimental
230 diets after 12-weeks

Parameters	Experimental diets					SEM	*P value*	
	DFSM-0	DFSM-3	DFSM-6	DFSM-9	DFSM-12		Linear	Quadratic
Hematocrit (%)	36.2	34.0	34.2	34.5	36.0	1.26	0.452	0.039
Hemoglobin (g dL^{-1})	12.5	11.0	11.3	11.5	12.6	1.52	0.623	0.235
MCHC (%)	34.5	34.2	33.0	33.4	34.9	0.96	0.956	0.527
Creatinine (mg dL^{-1})	0.42	0.41	0.40	0.41	0.39	0.02	0.265	0.985
Cholesterol (mg dL^{-1})	218.0	206.7	208.9	208.9	208.6	2.1	0.043	0.971
ALP (U L^{-1})	32.6	28.4	25.9	24.0	23.9	0.95	0.856	0.023
GPT (U L^{-1})	14.4	8.9	8.0	5.4	5.2	0.05	0.018	0.638
GOT (U L^{-1})	279.9	153.4	90.9	81.8	68.1	1.56	0.028	0.568

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