The effect of defatted black soldier fly (Hermentia illucens) meal inclusion in the formulated feed on the pellet stability at different salinity levels

Ishaaq Saputra1,* and Ravi Fotedar2

1Faculty of Engineering and Sciences, Curtin University, CDT 250, 98009 Miri Sarawak, Malaysia
2School of Molecular and Life Sciences, Curtin University, 1 Turner Avenue, Bentley, WA 6102, Australia

*Corresponding author: ishaaq.saputra@postgrad.curtin.edu.my

Abstract. Black soldier fly (Hermentia illucens) meal has become a promising alternative substitution of fishmeal as protein resources in aquafeeds. However, the effect of defatted BSF meal inclusion on the juvenile lobster feed stability have not been assessed. The present study is aimed to evaluate the effects of the inclusion of defatted BSF meal (25, 35 and 50 % replacement), the salinity level of seawater (30 and 35 ppt) and the immersion time (15, 30, 60, 90 and 120 minutes) on the pellet stability of formulated feed for juvenile lobster. The pellet stability was determined by calculate the dry matter loss of the feed (wet durability test). Results has shown that the dry matter loss of the feed ranged from 3.17 ± 0.03 % to 4.87 ± 0.26 % for the salinity of 30 ppt, and 3.27 ± 0.04 % to 4.46 ± 0.66 % for the salinity of 35 ppt. The dry matter loss was affected by the levels of defatted BSF inclusion level, the salinity, and the immersion time. The means of dry matter loss in formulated feeds were conversely affected by the salinity level and consistent in all formulated feeds. The means of dry matter loss in BSF25, BSF35 and BSF50 feed were significantly affected by the immersion time (P<0.05). At the salinity 30 ppt, significant differences of dry matter loss were observed after 120 minutes of immersion. Meanwhile, at 35 ppt the significant differences of dry matter loss were observed after 60 minutes after immersion. Significant differences (P<0.05) of dry matter loss were observed in the feed with the inclusion level of BSF of more than 25%. In conclusion the dry matter loss of formulated feeds for juvenile lobster is affected by the inclusion level of the defatted BSF, the salinity levels and the immersion times at acceptable level.

Keywords: BSF, feed, immersion, salinity

1. Introduction
Global aquaculture is progressing and supported by several factors such as the development of aquaculture methods, adoption of the advanced technology, and the application of a sustainable approach [1]. One of the main elements that are important in the culture of fish or shrimp is the availability of artificial feed. Artificial feed is critical in fish or crustacean farming activities because
feed is a source of nutrition for the growth and development of cultured fish or shrimp [2]. High quality and suitable artificial feed for certain fish species will result in optimal fish production. The suitable artificial feed should have sufficient nutrition to meet the requirement of fish development, and also has superior physical properties to minimize deterioration of water quality and maximize feed efficiency. Therefore, it is necessary to test the artificial feed, including the stability of the artificial feed in water using various methods [3].

The evaluation of formulated feed stability in the water is one of the key factors to do, to determine the quality of feed. The level of stability of feed in water can determine the amount of nutrients that can be consumed by fish so that the purpose of using artificial feed can be achieved [4, 5]. If the feed is decomposed too quickly in the water, the nutrients may leach to the water body and the optimum nutrition in the feeds for the fish become inadequate for growth. This event also can decrease the quality of the aquaculture water. A proper formulation and ingredients are needed so that the formulated feeds have physical properties which minimize nutrition leach in water at a certain time. So, the quality of the formulated feeds is as expected in the formulation. Since decades, the aquaculture industry put the fishmeal as a valuable resource for their roles in aquafeeds. Its physical and chemical properties combine with the ability in providing micronutrients including essential amino acids and fatty acids for fishes, make fishmeal becomes the main ingredient in every aquafeeds formulation [6]. However, the use of alternative ingredients instead of fishmeal began to be considered since massive use of fishmeal in aquaculture industry raises the concern about the cost production and environmental issues.

Black Soldier Fly (Hermetia illucens) or BSF has recently become a promising candidate for the protein source, substitutes the fishmeal in formulated feeds for fish and shrimp [7-9]. It is massively produced together with other insects such as Tenebrio molitor to fulfil the demand of alternative protein ingredients in aquaculture [10]. The application of BSF meal in the formulated feed has been reported to be suitable as fishmeal substitutes in salmon aquaculture [11-13], catfish [14], tilapia [15, 16] and crustaceans [10, 17]. Although the BSF have been successfully applied in several aquaculture species aforementioned, its application in spiny lobster aquaculture is scant. The work on the fishmeal substitution with BSF in spiny lobster is undergoing [18] and preliminary study of the formulated feed is required. Apart from the superiority of the BSF meal in terms of nutritional value, the using of alternative protein sources as a substitute for fishmeal in formulated feeds will certainly resulted on changes in the physical character of the feed [19, 20]. The changes of the composition of the feed was reported to have effect on the feed stability [4], durability [21] and palatability [22].

Since the studies on the use of the defatted BSF meals as the main protein source in the formulated feed for juvenile lobster are not available, the preliminary study of the effect of the inclusion of defatted BSF meal on feed stability has also not been studied. In addition, lobster-rearing media in the form of seawater may have a fluctuating salinity value which will have a different impact on the stability of the feed in the water. Therefore, it is necessary to conduct preliminary research to evaluate the effect of fish meal substitution using defatted BSF meal on dry matter loss of the formulated feed at different replacement levels, salinity levels of seawater and the time immersion.

2. Material and methods

2.1. Formulated feed preparation and experimental setup.

Black Formulated feed was made according to the number of ingredients specified in Table 1. Before use, each of the feed ingredients which are in form of powder, were filtered to produce uniform size using a special 1 mm sieve (Retch Test, Germany). After that, all the components of the material were weighed with a calibrated digital scale (OHAUS PA214C, US) with an accuracy of 0.01 g. All feed ingredients were then mixed and stirred until evenly distributed. The dough was then added with a small amount of distilled water to form a dough that is ready to be processed using an automatic noodle machine (Oxone OX-861N, Indonesia) with diameter size of 2 mm. The feed that is still in the form of noodles were then dried at 105°C for 16 hrs using air oven (Carbolite X50, US). After the completion
of drying process, the feed-noodle were then cut into pieces (3-4 mm length) and then were stored in the freezer (Midea HS-390CK, Indonesia) at -20 °C until it is ready for use.

Table 1 Ingredients of formulated feeds consisting of 0, 25, 35 and 50% of defatted BSF meal, replacing fish meal. The value displayed is as g/100 g of ingredients.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>0</th>
<th>25</th>
<th>35</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>64.8</td>
<td>48.6</td>
<td>42</td>
<td>32.3</td>
</tr>
<tr>
<td>Defatted Black Soldier Fly meal²</td>
<td>0</td>
<td>16</td>
<td>22.4</td>
<td>32.4</td>
</tr>
<tr>
<td>Astaxanthin (8%)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Binder (inert)</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Casein³</td>
<td>19.1</td>
<td>19.1</td>
<td>19.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>6.5</td>
</tr>
<tr>
<td>Corn starch</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Fish oil</td>
<td>1.3</td>
<td>1.5</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Vitamin premixª</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Lecithin (from soy)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Trace mineral premixª</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

ªVitamin mix contains retinol (900 IU/kg), vitamin C (200 mg/kg), D3 (200 IU/kg), menadione (10 mg/kg), vitamin E (100 mg/kg), vitamin B (1 g/kg), B8 (100 mg/kg), B1 (15 mg/kg), B2 (20 mg/kg), B6 (15 mg/kg), B5 (50 mg/kg), nicotinic acid (75 mg/kg), biotin (0.5 mg/kg), B12 (0.05 mg/kg), folic acid (5 mg/kg). Mineral mix contains cobalt (0.5 mg/kg), copper (5 mg/kg), iron (50 mg/kg), KI (4 mg/kg), chromium chloride (0.1 mg/kg), magnesium (150 mg/g), manganese (25 mg/kg), selenite (0.1 mg/kg), and zinc (100 mg/kg).
²Defatted BSF meal (Protein 55%, crude lipid 9%, carbohydrate 6% and ash 10%)
³Sodium caseinate, Friesland Campina-The Netherlands

In this preliminary study, four types of feeds were made based on the inclusion level of defatted BSF meal in the feed with compositions of 0, 25, 35 and 50% of the total replacement of fish meal. Then each type of feed was coded as BSF0, BSF25, BSF35 and BSF50. To calculate the level of feed stability in water, a water stability test was carried out on the four types of feeds by calculating the amount of dry matter content left in the feed after being dissolved into two salinity level (30 and 35 ppt) for 15, 30, 60, and 120 minutes.
2.2. Proximate analysis

The formulated feeds were analysed proximately according to an established protocol. The protein content of the feed was calculated based on the nitrogen content x 6.25 (Kjeldahl method) and carried out accordingly [23]. As for the ash content, it is calculated based on the residual burning of the sample at a temperature of 600°C for 4 hours using furnace. The determination of moisture content was performed using the oven method at full power at 600 watts for 120 s [24]. The crude lipid was measured using Soxhlet method [25].

$$\text{Dry matter loss} = 100 - \frac{W_t \times (1 - r) - W_o}{W_o \times (1 - r)} \times 100$$

where: $W_t$ = the initial dry weight of samples, $W_o$ = the final dry weight of samples, $r$ = the moisture content of the samples.

Table 2 Proximate composition of formulated feed with different inclusion levels of defatted BSF meal

<table>
<thead>
<tr>
<th>Proximate composition</th>
<th>Feed</th>
<th>BSF0</th>
<th>BSF25</th>
<th>BSF35</th>
<th>BSF50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (%)</td>
<td></td>
<td>84.79</td>
<td>88.21</td>
<td>87.15</td>
<td>88.80</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td></td>
<td>8.80</td>
<td>8.50</td>
<td>8.39</td>
<td>8.21</td>
</tr>
<tr>
<td>Ash (%)</td>
<td></td>
<td>8.8</td>
<td>8.48</td>
<td>8.35</td>
<td>8.20</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td></td>
<td>56.74</td>
<td>55.76</td>
<td>54.90</td>
<td>56.67</td>
</tr>
<tr>
<td>Crude lipid (%)</td>
<td></td>
<td>3.18</td>
<td>3.50</td>
<td>4.35</td>
<td>4.56</td>
</tr>
</tbody>
</table>

2.3. Pallet stability test

The pellet stability was determined using wet durability test of the feed according to [4] with modification. It was performed at two salinity level of seawater, 30 ppt and 35 ppt. To obtain the salinity of 30 ppt, the seawater stock having the salinity of 35 ppt were diluted with fresh water until it reached 30 ppt. The salinity was measured using a portable hand refractometer (ATG, China). From each type of feed, approximately 2 g of samples were taken and weighed using a digital scale (OHAUS PA214C, USA) until 3 decimal places and then stored in a 10 ml plastic cup. There are 30 samples in total for each type of feed to provide sufficient triplicate of five immersion time and two salinities treatments. As much as 5 ml of seawater from the stock was subsequently added to every cup. After adding seawater, the samples were allowed to immerse for 15, 30, 60, 90 and 120 minutes. After the completion of the immersion process, the mixed sample and water in the beaker were then filtered using filter paper to remove the water from the samples. After that, the samples were dried for 30 minutes at 105°C using an oven (Carbolite X50, US). At the end of the drying process, the dry matter of each sample was re-weighed to obtain sample weight. The amount of dry matter lost in the feed is calculated by an established protocol [4] using the following formula:

$$\text{Dry matter loss} = 100 - \frac{W_t \times (1 - r) - W_o}{W_o \times (1 - r)} \times 100$$

2.4. Statistical analysis

All values are presented as means ± SE of dry matter of the tested feed. A one-way ANOVA was performed to evaluate the effect of defatted BSF meal inclusion on the dry matter loss at different immersion times and salinity levels. An independent paired test was performed to evaluate the differences in the dry matter loss between salinity levels of feed type. All data were taken into normality and homogeneity of variance test to see the quality of collected data. The statistical analysis was
performed at a confidence level of 95% using SPSS software version 25.0 (Illinois, USA). A Turkey HSD post hoc test was performed to see the significant differences at P<0.05 among the treatments.

3. Results
In general, the dry matter loss of formulated feed showed a general trend of a lower dry matter loss when exposed to a higher salinity Figure 1. The dry matter loss of the formulated feed at salinity 30 and 35 ppt ranged between 3.17 to 4.87% and 3.27 to 4.46%, respectively. A paired sample t-test indicated that feed with the inclusion of defatted BSF meal significantly reduces the dry matter loss of the feed at higher salinity. In contrast, there was no significant differences in the formulated feed without defatted BSF meal at different salinity level.

![Figure 1 The average of total dry matter loss (%) of formulated feeds exposed to different time immersion and salinity level](image)

The dry matter loss of formulated feed was significantly different at a salinity of 30 among the BSF inclusion (P<0.05). In contrast, the dry matter loss at 35 ppt is the same (P>0.05) (Figure 1). A significant dry matter loss among formulated feed was observed in 60 minutes of immersion at both salinity levels. In addition, the inclusion of defatted BSF meal in the feed significantly affects the dry matter loss after 120 minutes of immersion in the salinity of 30 ppt (Table 3). In terms of dry matter loss of formulated feed following a certain level of immersion time, there was a general trend that the dry matter loss of formulated feed increases with the increase of time immersion at all salinity levels. At the salinity of 30 ppt, the inclusion of BSF meal in the feed increases the mean of dry matter loss significantly (P<0.05). Meanwhile, at the salinity of 35 ppt, significant dry matter loss occurs in feed with 35 and 50% of BSF meal.

![Figure 1 The average of total dry matter loss (%) of formulated feeds exposed to different time immersion and salinity level](image)

![Table 3 Total dry matter loss (%) of formulated feed under different salinity levels and immersion time. Data in the similar column with different superscripts (1,2) and data in the similar row with different superscripts (a,b) represent significant differences at P<0.05.](image)

<table>
<thead>
<tr>
<th>Immersion time (minutes)</th>
<th>Feed types</th>
<th>BSF0</th>
<th>BSF25</th>
<th>BSF35</th>
<th>BSF50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity 30 %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>3.41±0.03</td>
<td>3.30±0.04</td>
<td>3.42±0.05</td>
<td>3.45±0.02</td>
</tr>
</tbody>
</table>

Table 3 Total dry matter loss (%) of formulated feed under different salinity levels and immersion time. Data in the similar column with different superscripts (1,2) and data in the similar row with different superscripts (a,b) represent significant differences at P<0.05.
30 3.37±0.17 3.25±0.05 3.81±0.37 3.47±0.03
60 3.57±0.04a 3.17±0.03a,b 3.72±0.14b,1 3.55±0.04b,1
90 3.83±0.18 3.55±0.07a 4.07±0.11b,1 3.54±0.07b
120 4.03±0.23a,b 3.68±0.11a,b,2 4.87±0.26b,1,2 4.08±0.04a,b,2

<table>
<thead>
<tr>
<th>Salinity 35 %o</th>
<th>15</th>
<th>3.49±0.09</th>
<th>3.36±0.04</th>
<th>3.48±0.02</th>
<th>3.55±0.04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>3.52±0.06</td>
<td>3.53±0.04</td>
<td>3.56±0.02</td>
<td>3.35±0.08</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>3.29±0.01a,b</td>
<td>3.27±0.04a</td>
<td>3.63±0.04c,d,1,2</td>
<td>3.49±0.06b,c,1,2</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>3.47±0.14</td>
<td>3.78±0.11</td>
<td>3.60±0.24b,1</td>
<td>3.52±0.02b</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3.68±0.02</td>
<td>4.46±0.66</td>
<td>4.20±0.22</td>
<td>3.74±0.06</td>
</tr>
</tbody>
</table>

4. Discussion
In fish nutritional research, the initial study of feed physical properties is important to ensure the optimum delivery of nutrition within the feed for the fish. Although studies on defatted BSF inclusion in fish feed are abundant [7-9, 26], the attention regarding pellet stability following fishmeal substitution using defatted BSF is scant. In the present work, the substitution of fishmeal with defatted BSF meal in the feed makes significant changes in the dry matter loss. The increase of the dry matter loss due to the fishmeal substitution indicates that fishmeal as the source of protein in the reference feed (BSF0) has superior physical properties compared to defatted BSF which led to the better feed stability. However, the amount of dry matter loss in the feed that received defatted BSF meal substitution (BSF25, BSF30 and BSF50) was still within the acceptable range and relatively small compared to several previous studies which focusing on the physical formulated feed properties for crustaceans [10, 19, 27-29].

Salinity is one of the water quality parameters which play important roles in every brackish or seawater aquaculture activities and become limiting factor of some crustacean species [30, 31]. In spiny lobster, it has been reported that the juvenile lobster achieved their optimal growth at the salinity of 35 ppt and the growth decrease along with the decrease of salinity level [32, 33]. Salinity seems to be important for lobster aquaculture and since the majority of spiny lobsters were cultured in an open floating cage, the risk of salinity fluctuation is unavoidable. Therefore, effect of salinity to the quality of aquafeeds are important. At the salinity of 30 ppt, all feed with the inclusion of BSF meal experienced a gradual increase in dry matter loss in line with the immersion time. This result is as expected since the BSF meal has been reported to have higher moisture and ash content, which may affect the final quality of the feed [10]. A similar event also happened to the formulated feed when exposed to a salinity of 35 ppt, where BSF35 and BSF50 feeds experienced an increase in the dry matter loss along with the length of time immersion in water. Of concern is that in the current study, the dry matter loss rate in feed exposed to higher salinity was lower when compared to feed exposed to low salinity. The same results have been also reported in artificial feeds made for vannamei shrimp, L. vannamei [29]. The formulated feed stability in the water have been reported to be affected significantly by the feed ingredients [4, 20, 27-29, 34], binders [5, 19] and also environmental factor such as salinity [29, 32].

In general, the duration of the immersion of formulated feed in the water is proportionally affected the dry matter loss of the formulated feed. This is because the feed will absorb the surrounding water causing a gradual breakdown of the feed. Several things can increase the level of durability of fish feed in water such as binders [5, 19]. This is because each binder has different characteristics from one to another. Binders such as carrageenan are known to have better adhesion than agar, gelatine and carboxymethylcellulose (CMC) which will result in maximum water durability [5]. In this study, a
significant difference in the dry matter loss of the feed was observed at immersion times of 60 and 120 minutes for 30 ppt salinity and 60 minutes for 35 ppt salinity. Meanwhile, BSF25 feed was known to have dry matter loss at 60 minutes of immersion at both salinity levels and 120 minutes at 30 ppt. The greatest dry matter loss occurred in BSF35 feed at 60 minutes immersion time and 35 ppt salinity with the rate of dry matter loss rate at 3.63%. However, it is still relatively low compared to shrimp feed supplemented with CMC with dry matter loss of 8-9% [19]. In addition, some commercial shrimp feeds was reported to have dry matter loss levels between 10.8 to 14.2% at salinity of 20 ppt [27]. The low dry matter loss in the present work may be due to the use of corn starch as a binder which improves the feed durability. Therefore, the maximum dry matter loss of the formulated feed in this study was within the acceptable level for fish feeds [35].

5. Conclusion
The dry matter loss of formulated feeds for juvenile lobster is affected by the inclusion level of the defatted BSF, the salinity levels and the immersion times at acceptable level. Based on the present study, formulated feed with defatted BSF meal at all replacement levels were suitable in terms of water stability at the salinity of 30 and 35 ppt. In addition, the present study also indicated that the time interval of feeding using the current feed is up to 120 minutes without any affect to the formulated feed quality. The results in this study allowed us to conclude that the dry matter loss of the formulated feed for juvenile lobster was affected by the inclusion of defatted BSF meal with respect to the salinity level and time immersion within the acceptable levels.

6. Acknowledgements
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References


