# Pre-treatment of Black Soldier Fly Larvae (BSFL) using neutral salt to improve protein digestibility of *Macrobrachium rosenbergii* feed

To cite this article: W Muhammad Amiruddin *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **842** 012071

# Pre-treatment of Black Soldier Fly Larvae (BSFL) using neutral salt to improve protein digestibility of *Macrobrachium rosenbergii* feed

**W Muhammad Amiruddin[1], A M Siti Zarin Sofia[1], S M Al-Amsyar[1], N D Rusli[1,2], K B Mat[1,2], S A Muhamad Sukri[1], M Mohd[1,2] and H C Harun[1,2*]**

[1]Department of Agriculture, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Malaysia
[2]Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600 Jeli, Malaysia
*Corresponding author: hasnita@umk.edu.my

**Abstract**. *Macrobrachium rosenbergii* (*M. rosenbergii*) is one of the economically driven freshwater aquaculture species in many countries. The production of *M. rosenbergii* has been increased over the year. Along with the growth of production of aquaculture species, feed production also rises. *Artemia* nauplii are the main live feed with partial replacement with egg custard. However, the application of live feed is costly. Black Soldier Fly larvae (BSFL) is a high potential source of protein that can be applied as *M. rosenbergii* larvae feed. Meanwhile, pre-treatment of BSFL using neutral salt (NaCl and KCl) is essential to improve the protein digestibility of *M. rosenbergii* larvae. This study aims to produce feed that can help in producing fast-growing and healthy *M. rosenbergii* larvae. Different percentages of salt were at 5%, 10%, and 15% were used for BSFL pre-treatment, while BSFL without the addition of salt was used as a control diet. The present study showed that pre-treated BSFL with 15% KCl had a higher protein decreased among the other treatments, followed by 10% KCl and 15% NaCl. In addition, egg custard with 100% BSFL has the highest crude protein and lipid content, which was higher than control (without BSFL), which has the lowest crude protein and lipid content. This study shows that the formulated egg custard has the optimum nutrient that meets the *M. rosenbergii* larvae requirement.

## 1. Introduction

Giant freshwater prawn or scientifically known as *Macrobrachium rosenbergii* belongs to the genus *Macrobrachium* and the largest genus in the *Palaemonidae* family. This freshwater aquaculture species is an important economically driven in many countries, including Asia and South America, due to its high market value [1]. In Malaysia, *M. rosenbergii* has shown a rapid increase and be predicted to continue as more farming efforts are initiated [2]. Along with the rapid growth of the aquaculture industry, there was an increase in feed production [3]. The efficient use of locally available food resources is the key for sustainable commercial production of aquaculture species, including *M. rosenbergii* [4]. Soybean meal, chicken waste meal and, fishmeal are examples of excellent protein sources for *M. rosenbergii* [5]. On the other hand, plant resources also are being used as one of the main ingredients in *M. rosenbergii* culture due to its source of proteins, carbohydrates, fats, amino acids, vitamins, and minerals such as moringa and turmeric [6,7]. *Artemia* nauplii and *Moina* are some examples of live foods used in the different larval stages. Application of the *Artemia* as live feed can be

replaced by egg custard or a combination of both [8]. These types of feed may be productive and reliable to use, but the price is high, which may be a disadvantage for the small farmers. Locally available ingredients are becoming a significant focus to reduce production costs. Search for alternative protein sources has increased recently to produce low cost, effective diets and high protein feed [9].

The insect has been used as feed for monogastric animals (e.g., poultry and fish). The fly larvae are recommended as the alternative feed due to their high animal protein intake [10]. One of the main species of fly larvae that have been used as animal feed is the black soldier fly (*Hermetia illicens*). BSFL production was recognised as a model system for reducing waste to produce protein [11]. The BSFL receives much attention due to its high levels of lipids and proteins, which can be used to feed fish and poultry [12]. BSFL also contains about 5% of calcium, much higher than many insect species [13]. Surendra *et al*. [14] stated that BSFL is well known as the best insect for bioconversion. One of the advantages of BSFL is that they can feed on many types of organic wastes and some studies show that BSFL can accept waste from both animal and plant origins. Even though it can consume a wide range of waste, the type of waste chosen can impact their development time [15].

Studies on the nutrition of *M. rosenbergii* have been increased significantly in recent years. *M. rosenbergii* at the larvae stage require higher protein and lipid than post-larvae and grow out prawns [16]. Protein has received more attention than other nutrients because it is the main nutrient requires in animal feed that important for growth and maintenance, and it is an expensive component in a diet. On the other hand, excessive levels of protein will increase the feed cost and nitrogenous waste. Therefore, it is vital to know the optimum protein level to reduce feed costs and water pollution. Determination of feed ingredients' digestibility is essential to be evaluated to ensure the efficiency of a low feed conversion ratio (FCR). Thus, the study aims to determine the potential application of treating BSFL using neutral salt to improve protein digestibility for *M. rosenbergii* larvae.

## 2. Materials and Method

### *2.1. The Black soldier fly (Hermetia illucens) larvae and defatting process*

A total of dried 2kg of Black soldier fly larvae (BSFL) powder was purchased from a local producer. Then, about 20g of BSFL powder was filled inside a piece of filter paper before depositing in thimble cellulose. The fat from the BSFL powder was extracted for 5 to 6 hours by using the Soxhlet method in which the Soxhlet apparatus was used. A total of 95% ethanol was used as an extractor to extract the BSFL power. After the defatting process, the defatted BSFL powder was dried.

### *2.2. Preparation of Standard curve using BSA Standard for Bradford Protein Assay*

A standard protein solution was prepared by using Bovine Serum Albumin (BSA) powder. The concentration of the solution was adjusted until it reaches a concentration of 1mg/mL. A 100µL micropipette has been used to make the standard concentration and was added in different microcentrifuge tubes. From the prepared standard concentration, 100µL of it was taken and mixed with 1mL of Bradford reagent. A vortex mixer was used to mix the mixture well, and the sample was rest for 5 minutes. After 5 minutes, the sample was poured into a cuvette before placing into a UV spectrophotometer detector. The wavelength used is 595nm, and the reading of the absorbance was recorded. Data of extinction against standard concentration was collected and analysed.

**Table 1.** Preparation of standard concentration.

| Standard concentration (mg/µL) | 1mg/mL BSA solution (µL) | Phosphate buffer solution (µL) |
|---|---|---|
| - | - | 500 |
| 25 | 12.5 | 487.5 |
| 50 | 25 | 475 |
| 75 | 37.5 | 462.5 |
| 100 | 50 | 450 |

### *2.3. Pre-treatment of BSFL with salt*

The defatted BSFL were pre-treated with different percentages of salt, i.e., 5%, 10%, and 15%. The defatted BSFL without additional salt was used as a control test diet. The mixture of defatted BSFL powder, salt (NaCl and KCl), and distilled water were mixed inside the conical flask, as in Table 2. Then, the conical flask that contained a mixture of BSFL, salt, and water was placed at an open shaker. The mixture was shaken for 48 hours at room temperature and 150 rpm.

**Table 2.** Preparation of test diet.

| Test diet | Composition |
|---|---|
| Control (0%) | 2g of defatted BSFL + 8mL of distilled water |
| 5% NaCl or KCl | 1.9g of defatted BSFL + 0.1g salt + 8mL of distilled water |
| 10% NaCl or KCl | 1.8g of defatted BSFL + 0.2g salt + 8mL of distilled water |
| 15% NaCl or KCl | 1.7g of defatted BSFL + 0.3g salt + 8mL of distilled water |

### *2.4. Bradford assay of Pre-treated BSFL*

After the shaking process, the mixture was poured into a falcon tube before centrifuging it (25 °C, 4000 rpm and 10 minutes) to separate the supernatant and the precipitate. Then that, the supernatant was transferred into a new falcon tube. 100µL of supernatant was mixed with 1mL of Bradford reagent in the microcentrifuge tube. A vortex mixer was used to mix the solution well, and then it was let to rest for 5 minutes. Then, the sample was poured into a cuvette for absorbance reading. A UV spectrophotometer detector was used to read the absorbance and the wavelength used is 595nm. The reading of the absorbance was recorded.

### *2.5. Egg custard formulation*

Eggs and skimmed milk were used to create the egg custard. A total of 5 egg custard formulations prepared (i.e., 0%, 25%, 50%, 75% and 100% of pre-treated BSFL). Powdered moringa and turmeric were used as additional ingredients. The ingredients were mixed by using a hand mixer until they became homogenised and steamed for 20 minutes. Then the egg custard was stored in a refrigerator overnight. After being cool overnight, a 300µm sieve was used to mesh the egg custard into a small particle that suits to feed *M. rosenbergii* larvae.

**Table 3.** Preparation of the formulated egg custard.

| Test diet | Egg (mL) | Skimmed milk (g) | Treated BSFL (g) | Moringa powder (g) | Tumeric powder (g) |
|---|---|---|---|---|---|
| Control EC (0%) | 13 | 20 | - | 0.2 | 0.2 |
| 25% EC BSFL | 13 | 15 | 5 | 0.2 | 0.2 |
| 50% EC BFSL | 13 | 10 | 10 | 0.2 | 0.2 |
| 75% EC BSFL | 13 | 5 | 15 | 0.2 | 0.2 |
| 100% EC BSFL | 13 | - | 20 | 0.2 | 0.2 |

### *2.6. Proximate analysis*

The proximate analysis is done for all samples to determine the nutritional composition. First, the BSFL nutritional composition was determined during untreated and pre-treated with 15% KCl. Then, another proximate analysis was done to determine the nutritional composition of formulated egg custard.

### *2.7. Statistical analysis*

All the collected data were analysed using one-way ANOVA available from Statistical Package for the Social Science (SPSS version 25) to find the significant difference between treatment group and

followed by Tukey post hoc test at the level of significance 5% ($p<0.05$). Data were presented as mean ± SEM.

## 3. Results and Discussion

### *3.1. Standard Curve for Bradford Protein Assay*

Bovine Serum Albumin (BSA) is a protein that has been widely used in biochemical applications due to its standard protein concentration, stability and low impact in biochemical reactions [17]. In the present study, the standard curve was successfully plotted using the BSA standard according to the absorbance value in Table 4.

Based on Figure 1, an equation of y=mX+c is obtained. The increase of BSA concentration shows that the trend line of the point also increases, which the $R^2$ is equal to 0.9726. The closer the $R^2$ value to 1.00 indicates that the curve is more accurate [18]. The equation obtained was used to quantify the pre-treated BSFL concentration.

**Table 4.** The absorbance of the BSA concentration at 595nm.

| Concentration of BSA (mg/µL) | Average Absorbance (595nm) | Absorbance (595nm) |
|---|---|---|
| 0 | 0 | 0 |
| 25 | 0.0878 | 0.0878 |
| 50 | 0.1263 | 0.1263 |
| 75 | 0.2536 | 0.2536 |
| 100 | 0.2921 | 0.2921 |

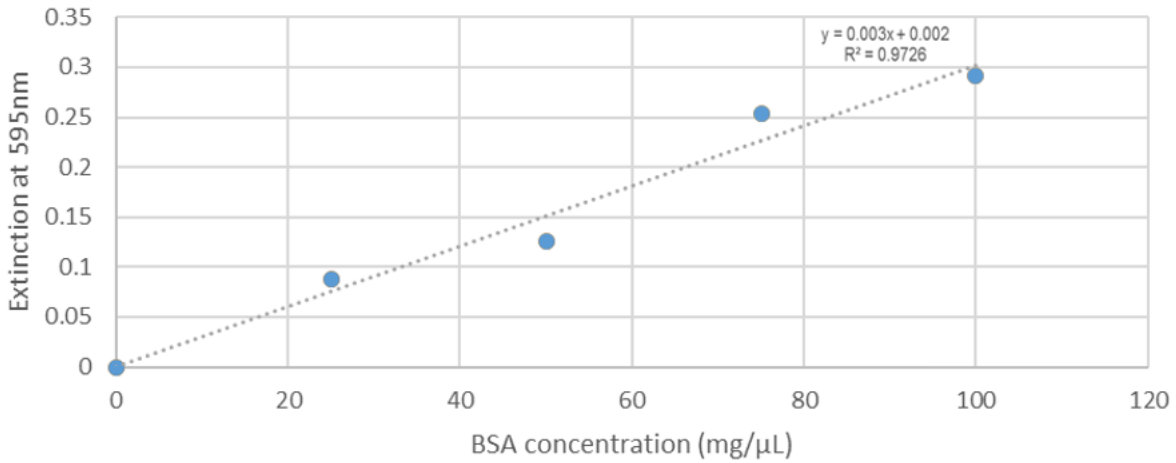


**Figure 1.** The extinction graph at 595nm against BSA concentration.

### *3.2. Protein decreased of Pre-treated BFSL*

By using the equation obtained from the standard curve of y=0.003x+0.002, the protein concentration of the control and pre-treated BSFL were obtained. Results from the present study show a significant protein decrease for all treatments (5% NaCl, 10% NaCl, 15% NaCl, 5% KCl, 10% KCl and 15% KCl). Table 5 shows that the application of 15% salt for both NaCl and KCl has the highest protein decreased, followed by 10% and 5%, respectively.

It can be concluded that the higher the concentration of the salt resulted in more protein decreased. The mean score of protein decreased is 10.65±0.005 for 5% NaCl, 13.93±0.003 for 10% NaCl and 17.34±0.01 for 15% NaCl. Meanwhile, the protein decreased is 10.65±0.007 for 5% KCl, 13.93±0.005 for 10% KCl, and 17.34±0.006 for 15% KCl, respectively. Protein denaturation can be described as changes in protein structure that cause biological activity loss. According to Sinha and Khare [19], salt

in the ionic form will link within protein moiety, resulting in surface hydration. However, they also stated that high salt concentration would increase the water surface tension, which causes the salt ions and the protein to compete for hydration. The salt will remove the essential layer of a water molecule from the protein surface, resulting in protein denaturing. Even though both 15% NaCl and KCl show the highest protein decreased between the same salt, 15% KCl shows the highest protein decreased compared to 15% NaCl.

**Table 5.** Protein decreased of pre-treated BSFL.

| | Protein decreased | |
|---|---|---|
| Test diet | NaCl | KCl |
| 5% | $10.65\pm0.005^{c}$ | $9.81\pm0.007^{b}$ |
| 10% | $13.93\pm0.003^{d}$ | $17.78\pm0.005^{f}$ |
| 15% | $17.34\pm0.01^{e}$ | $21.21\pm0.006^{g}$ |

[ab] means with different superscripts in a row is significantly different ($p<0.05$)

*3.3. Biochemical Composition of Untreated and Pre-treated BFSL and different BSFL feed formulations*

*3.3.1. Biochemical Composition of Untreated and Pre-treated BFSL.* The proximate analysis was conducted between the untreated BFSL (Control, 0%) with the most protein decreased pre-treated BSFL with 15% KCl. Table 6 shows the proximate composition of untreated BSFL and pre-treated BSFL with 15% KCl.

**Table 6.** Proximate composition of untreated BSFL and pre-treated BSFL with 15% KCl.

| Parameter | Test diet | |
|---|---|---|
| | Untreated BSFL (Control, 0%) | Pre-treated BSFL with 15% KCl |
| Crude protein | $52.05\pm0.31^{f}$ | $46.01\pm0.05^{e}$ |
| Crude fat | $0.11\pm0.01^{a}$ | $0.28\pm0.01^{a}$ |
| Crude fibre | $19.26\pm0.40^{g}$ | $17.12\pm0.17^{f}$ |
| Ash | $15.01\pm0.20^{e}$ | $26.58\pm0.17^{g}$ |
| Moisture | $11.55\pm0.42^{b}$ | $7.02\pm0.38^{a}$ |

[ab] means with different superscripts in a row is significantly different ($p<0.05$)

As shown in Table 6, the crude protein of pre-treated BSFL with 15% KCl is significant ($p<0.05$), which lower than untreated BSFL. This occurrence happened because the additional 15% KCl in salt concentration will undergo denaturation of protein. According to Shumo *et al.* [20], the crude protein of the BSFL can be up to 50%, and as for Dossey *et al.* [15], the crude protein of BSFL are 47%. Un-defatted BSFL usually contains up to 35% of lipid or crude fat. Since the test diet used BSFL that already undergo defatting process via the Soxhlet method, the drastic decrease of crude fat can be seen between un-defatted BSFL and defatted BSFL. From the result, the percentage of crude fat of untreated BSFL is $0.11\pm0.01$, and for pre-treated BSFL with 15% KCl is $0.28\pm0.01$. The difference between percentages of crude fat may occur during the defatting process. Some of the factor that might contribute to this can be the different temperature between the Soxhlet apparatus since the defatting process was conducted using multiple set of Soxhlet apparatus. Next, for the crude fiber, the percentage of crude fibre of the untreated BSFL is $19.26\pm0.56$ and $17.12\pm0.23$ for pre-treated BSFL with 15% KCl. This shows that the percentage of the crude fibre of the untreated BSFL is significant ($p<0.05$), which higher compared to pre-treated BSFL. Some of the studies on the effects of salt on wheat showed that, the higher the salt concentration result in decreased of crude fibre content [21]. Then for ash content, untreated BSFL ash content is $15.01\pm0.28$ and for pre-treated BSFL is $26.58\pm0.23$. The result shows that the ash content of

the pre-treated BSFL is significant ($p<0.05$), which higher than untreated BSFL. Research done by Salman [22] stated that the ash content between regular commercial feed was lower (9.1%) compared to commercial feed that content medium salt concentration (19.2%). It shows that adding salt will significantly ($p<0.05$) increase the ash content of the feed. Lastly, the moisture content of untreated BSFL is significant ($p<0.05$), higher than pre-treated BSFL.

*3.3.2. Biochemical Composition of Formulated Egg Custard.* A total of five different formulations of egg custard (0%, 25%, 50%, 75%, and 100% of pre-treated BSFL) have been undergoing proximate analysis. Pre-treated BSFL with 15% have been used as a substitution for skimmed milk powder. Table 7 shows the egg custard formulation proximate composition.

**Table 7.** Proximate composition of egg custard formulations.

| Parameter | Test diet | | | | |
|---|---|---|---|---|---|
| | EC without BSFL | EC with 25% BSFL | EC with 50% BSFL | EC with 75% BSFL | EC with 100% BSFL |
| Crude protein | $23.36\pm0.34^{a}$ | $26.00\pm0.09^{b}$ | $28.37\pm0.18^{c}$ | $29.50\pm0.26^{c}$ | $32.00\pm0.22^{d}$ |
| Crude fat | $1.10\pm0.02^{b}$ | $2.14\pm0.04^{c}$ | $2.11\pm0.13^{c}$ | $2.83\pm0.09^{d}$ | $3.68\pm0.11^{e}$ |
| Crude fibre | $1.14\pm0.04^{a}$ | $3.62\pm0.05^{b}$ | $6.62\pm0.19^{c}$ | $10.85\pm0.27^{d}$ | $14.12\pm0.28^{e}$ |
| Ash | $4.77\pm0.03^{a}$ | $7.38\pm0.01^{b}$ | $10.30\pm0.02^{c}$ | $13.67\pm0.10^{d}$ | $16.35\pm0.04^{f}$ |
| Moisture | $28.55\pm0.16^{c}$ | $30.12\pm0.21^{cd}$ | $30.67\pm0.15^{d}$ | $34.39\pm0.34^{e}$ | $35.70\pm0.29^{e}$ |

[ab] means with different superscripts in a row is significantly different ($p<0.05$)

From the proximate composition, all parameters show significant results ($p<0.05$), which the highest for egg custard with 100% BSFL test diet, whereas the EC without BSFL (Control) shows the lowest. The parameter increased along with the increased BSFL used; meanwhile, along with the increased BSFL used, the amount of skimmed milk powder used in making EC also was decreased. The nutrient requirement of *M. rosenbergii* is various according to their growth stage. In terms of protein requirement, broodstock of *M. rosenbergii* requires about 38% to 40%, juveniles require 35% to 37%, and adults need 28% to 30% of protein, respectively [23]. Based on Table 6, egg custard with 100% BSFL as a percentage of crude protein of 32.00±0.31, which can provide nutrients for almost the *M. rosenbergii* growth stages. Besides protein requirement, crude fat or lipid requirements for all growth stages of *M. rosenbergii* are 3% to 7%. The result shows that egg custard with 100% BSFL can supply lipid for *M. rosenbergii* since the result shows 3.68±0.16% of crude fat. A standard egg custard formulation cannot provide lipid for *M. rosenbergii* since the crude fat content (1.10±0.03) does not reach their requirement. Without skimmed milk powder, the formulated egg custard can provide nutrients for *M. rosenbergii,* which can help farmers in the future.

## 4. Conclusion

The pre-treatment of Black soldier fly larvae using different concentrations of NaCl and KCl, which then applied to improve egg custard formulation, showed high potential to be used as *M. rosenbergii* larvae feed. A high concentration of salt helps in protein denaturation, thus makes the protein easy to digest. The crude protein of the formulated egg custard is much higher compared to the regular egg custard. The combination of salt with BSFL can help better digestion and absorption of many nutrients essential for *M. rosenbergii* larvae growth.

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