

PAPER • OPEN ACCESS

Optimisation of treated Black Soldier Fly Larvae (BSFL) using acidic salt to improve protein digestibility of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae

To cite this article: H C Harun *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **842** 012067

View the [article online](#) for updates and enhancements.

You may also like

- [Optimisation of biodiesel production of Black Soldier Fly larvae rearing on restaurant kitchen waste](#)
S Ishak, A Kamari, S N M Yusoff *et al.*
- [Optimisation of Protease-Treated Black Soldier Fly Larvae \(BSFL\) using Response Surface Methodology \(RSM\) for Broiler Feed](#)
Nurul Athirah Mohd Zuki, Siti Sarah Mohamad Kamal, Nik Kamaruzaman Nik Qalbee *et al.*
- [Study on the Adsorption Properties of Different Insect Feces on Cu²⁺ and Cd²⁺](#)
Mingfu Zhang, Zhijuan Tang, Yi Zhao *et al.*

Optimisation of treated Black Soldier Fly Larvae (BSFL) using acidic salt to improve protein digestibility of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae

Harun H C^{1,2*}, Muhammad Amiruddin W¹, Leng O S¹, Abdul Malik S Z¹, Yee L S¹, Rusli N D^{1,2}, Mat K B^{1,2}, Mohd M^{1,2}, Sukri S A M^{1,2} and Syed M Al-Amsyar¹

¹Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia.

²Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia

*Corresponding author: hasnita@umk.edu.my

Abstract. The high cost of *Artemia* sp. and the shorter shelf life of egg custard as the major aquaculture feed for *Macrobrachium rosenbergii* are giving burden to aquaculture farmers in financial support and labour force. In most studies, BSFL has proved to successfully replace commercial animal feed which BSFL has similar amino acid (AA) profile as fish meal (FM). Application of pre-treated BSFL introduced as an alternative feed for *M. rosenbergii* might be potential. Pre-treatment of BSFL was carried out by mixing 2 different types of acidic salt, potassium dihydrogen phosphate (MKP), KH_2PO_4 and sodium dihydrogen phosphate (MSP), NaH_2PO_4 . Three different concentrations of each acidic salt were used to treat BSFL (5 %, 10 % and 15 %). Treated BSFL which has the highest percentage of protein decreases were selected and further identified biochemical composition through proximate analysis. Next, treated BSFL were used to partially replaced skimmed milk powder with 25% BSFL, 50% BSFL, 75% BSFL and 100% BSFL as protein source and formulated with grade A chicken egg, 1% moringa and 1% turmeric. Physical properties on treated diets and formulated egg custards are identity to determine the suitable feed for *M. rosenbergii* larvae. BSFL which treated with 15% KH_2PO_4 has highest protein decrease which is 17.39 % compared to BSFL that treated with 5% NaH_2PO_4 , 10% NaH_2PO_4 , 15% NaH_2PO_4 , 5% KH_2PO_4 and 10% KH_2PO_4 (5.16 %, 12.98 %, 16.72 %, 10.38 % and 15.22 %). In addition, egg custard with 100% BSFL has higher protein content which is 33.88 % compared to egg custard, egg custard with 25% BSFL, egg custard with 50% BSFL and egg custard with 75% BSFL (23.36 %, 27.88 %, 30.21 % and 32.66 %). It shows that the potential application of treated BSFL provides optimum nutrients to *M. rosenbergii* to replace current commercial feed.

1. Introduction

Macrobrachium rosenbergii is among commercially cultivated species not only in its natural habitat but beyond. It is known that approximately 70% of the aquaculture production cost is on the feed itself. However, the high dependency on the imported supplies of commercial feed that is high in price has limits the expansion of this industry. For instance, in the recent years, the prices for fish meal (FM) is reported to be increased to about twofold [1].



Current practice in *M. rosenbergii* larvae culture is by utilising live feed as the main feed resources, *Artemia* sp. (Brine shrimp) [2],[3]. However, its expensive price has urged the farmers to find alternative feed such as egg custard [4]. Egg custard is used to partially replace *Artemia* sp. In addition, different larval stages of *M. rosenbergii* require a wide variety of feed to fulfil their growth requirement and performance as feeding behaviours and digestive system tract has changes in different stages (FAO, 2020).

To reduce financial burden and improves protein digestibility of aquatic animal, scientific knowledge on insects as a meal and food has been growing exponentially in the last 5 years [5]. Black Soldier Fly Larvae (BSFL) (*Hermetia illucens*) is a member of family *Stratiomyidae* that commonly used to treat organic waste. In recent years, the application of BSFL as feedstuff is increasing gradually as they contain high protein levels which is in the ranges of 37 % to 63 % [6][7][8].

A study from [9] and [10] reported that defatted insect meal contains 83 % of crude protein (CP) even the CP level in most insects ranges from 40 % to 63 %. High digestible feed has provided better nutrient absorption of animals for performing biological functions and maintaining optimal health and well-being. Salt, one of the essential mineral elements to improve digestibility and palatability of feed is requires for normal functioning of animal and plant bodies and improving their growth performance [11]. The present study aims to investigate the potential application of acidic salt to pre-treat the BSFL to improve protein digestibility in the Giant Freshwater Prawn larvae.

2. Pre-treatment of BSFL using Acidic Acid

2.1. Preparation of Protein Standard Curve

Bovine Serum Albumin (BSA) was used to prepare the protein standard curve using standard method.

2.2. Defatting of Black Soldier Fly Larvae

BSFL powder was supplied by Ori Biotechnology Sdn Bhd. BSFL was sieved using 300-micrometre test sieve to get the desired texture for feeding *M. rosenbergii* at the larval stage. Fat of BSFL were extracted for 6 hours by Soxhlet extraction with 20 g of BSFL and 300 mL of 95 % of ethanol. After 6 hours, the collected oil was discarded. Next, BSFL powder was taken out and air dried in the open air for more than 48 hours to remove moisture and ethanol.

2.3. Experimental Diet

Seven different treated diets were prepared from defatted BSFL powder and 2 different types of acidic salt (MSP and MKP) with respectively salt content (5 %, 10 % and 15 %). Treated diets were created with 6 different acidic salt treatments (N₁, N₂, N₃, K₁, K₂ and K₃). Control diet, without any salt treatment, C (0 %) was included. Each treatment and control were run in triplicates. Table 1 showed the preparation of the treated diet in the experiment.

Table 1. Preparation of experimental diet using different salt concentration.

Treatment		Experimental Diet Composition
MSP (NaH ₂ PO ₄)	MKP (KH ₂ PO ₄)	
C		2 g BSFL powder and 8 mL distilled water
N ₁	K ₁	1.9 g BSFL powder, 0.1 g acidic salt and 8 mL distilled water
N ₂	K ₂	1.8 g BSFL powder, 0.2 g acidic salt and 8 mL distilled water
N ₃	K ₃	1.7 g BSFL powder, 0.3 g acidic salt and 8 mL distilled water

2.4. Bradford Protein Assay

A total of 2 mL of control diet, C were transferred into micro-centrifuge tube using dropper. Next, clear supernatant was obtained after centrifuged at room temperature for 30 minutes at 4000 r.p.m. The clear supernatant was transferred into another micro-centrifuge tube to assay protein with Bradford reagent. 100 μ L of supernatant was pipetted from control diet, C inserted into micro-centrifuge tube followed by 1 mL of Bradford reagent. The mixture was vortexed and incubated for 5 minutes. Next, the absorbance value of supernatant was detected by Shimadzu UV-1900 UV-Vis spectrophotometer at 595 nm for determination on the protein concentration of control diet, C. The result was obtained and recorded. The steps were repeated and replaced with N₁, N₂, N₃, K₁, K₂ and K₃.

2.5. Chemical composition

Treated BSFL was oven dried for 24 hours at 55 °C to 60 °C to remove moisture. Four different formulations of egg custard were prepared from treated BSFL powder with respective content (25 %, 50 %, 75 % and 100 %) whereby the usage of skim milk powder was reduced from 75 %, 50 %, 25 % and 0 %, respectively. In addition, 1 % of moringa and 1 % of turmeric were weighed and added into the mixture. Table 2 showed the ingredient of feed formulation in the experiment.

Table 2. Preparation of Formulated Egg Custard.

Formulation	Formulated Diet Composition
EC ₁	20 g skimmed milk powder, 13 mL grade A chicken egg, 0.2 g moringa and 0.2 g turmeric
EC ₂	5 g treated BSFL powder, 15 g skimmed milk powder, 13 mL grade A chicken egg, 0.2 g moringa and 0.2 g turmeric
EC ₃	10 g treated BSFL powder, 10 g skimmed milk powder, 13 mL grade A chicken egg, 0.2 g moringa and 0.2 g turmeric
EC ₄	15 g treated BSFL powder, 5 g skimmed milk powder, 13 mL of grade A chicken egg, 0.2 g moringa and 0.2 g turmeric
EC ₅	20 g treated BSFL powder, 13 mL of grade A chicken egg, 0.2 g moringa and 0.2 g turmeric

Formulation (Control egg custard, EC₁) is prepared from 20 g of skimmed milk powder, 13 mL of grade A chicken egg, 0.2 g moringa and 0.2 g turmeric. Total weight of 20 g of formulated egg custard obtained from 25 %, 50 %, 75 % and 100 % of treated BSFL powder with 5 g, 10 g, 15 g and 20 g and mixed with 15 g, 10 g, 5 g and 0 g of skimmed milk powder, respectively. Next, 0.2 g moringa and 0.2 g turmeric is added and mixed well with treated BSFL and skimmed milk powder before adding chicken egg. EC₂, EC₃, EC₄ and EC₅ were obtained after 13 mL of grade A chicken egg was poured little by little and well mixed. Every formulation (EC₁, EC₂, EC₃, EC₄ and EC₅) was steamed for 20 minutes. Formulation, EC₁, EC₂, EC₃, EC₄ and EC₅ was sieved through 300-micrometre test sieve and kept in a refrigerator for later use.

2.6. Proximate analysis

Chemical composition (crude protein, crude fat, crude fibre, ash and moisture) of each treatment groups (before and after treated with acidic salt) and control were determined using proximate analysis. The analysis was carried out using AOAC (1990) procedures. Each sample was run in triplicates.

2.7. Physical test

Four analysis which were colouration, hardness, sinking velocity and bulk density were carried out on all samples in triplicates to determine the physical characteristic of formulated feeds.

2.8. Data analysis

The data obtained from the experiments were analysed using one-way ANOVA and Tukey post hoc test ($p < 0.05$) available from Statistical Package for the Social Sciences (SPSS) version 25. The result was presented as mean \pm SEM of the replicated treatments for each data.

3. Result and Discussion

3.1. Effect of Pre-Treatment on the BSFL

The percentage of protein decrease of six different treatments were calculated. As shown in Table 3, percentage of protein decrease in BSFL was decreased gradually when the more the amount of acidic salt added. The protein decreases of BSFL significantly different ($P < 0.05$) in all treatments. K_3 shows higher mean percentage of protein decrease ($17.39 \pm 0.0017\%$) compared to N_1 , N_2 , N_3 , K_1 and K_2 whereby $5.16 \pm 0.0017\%$, $12.98 \pm 0.0029\%$, $16.72 \pm 0.0034\%$, $10.38 \pm 0.0051\%$ and $15.22 \pm 0.0029\%$, respectively. Addition of salt help to denature the protein content of BSFL because formation on the hydration of surface from the ionic form of salt associate with opposite charges within the protein moiety [12]. Moreover, protein denaturing occurs while salt strip off an essential layer of water molecules from the protein surface [12]. Although the same amount of acidic salt was added, the percentage of protein decreases with KH_2PO_4 treatment is higher compared to NaH_2PO_4 treatment. This could be due to the functional properties and chemical properties of potassium, K and sodium, Na gave different effects on treatment. Thus, K_3 was chosen for further use in feed formulation with different inclusion rates of BSFL.

Table 3. Mean score for Percentage of Protein Decrease of Respectively Treatment After Bradford Protein Assay.

Test Diet	Protein Decrease, %
N_1	5.16 ± 0.0017^b
N_2	12.98 ± 0.0029^c
N_3	16.72 ± 0.0034^d
K_1	10.38 ± 0.0051^e
K_2	15.22 ± 0.0029^f
K_3	17.39 ± 0.0017^g

Mean within column with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$.

3.2. Proximate Composition of Experimental Feed

The biochemical composition of treated diets and formulated egg custard is reported in Table 4. Biochemical compositions (crude protein, crude fat, crude fibre, ash and moisture) shown significantly different ($P < 0.05$) in all treated diets and formulated egg custard.

Table 4. Mean Score for Biochemical Composition of Treated Diets and Formulated Egg Custards with Crude Protein, Crude Fat, Crude Fibre, Ash and Moisture.

Parameter	Treatment & Formulation						
	EC_1	Defatted BSFL	K_3	EC_2	EC_3	EC_4	EC_5
Crude Protein, %	23.36 ± 0.34^a	52.05 ± 0.31^e	51.30 ± 0.26^e	27.88 ± 0.22^b	30.21 ± 0.09^c	32.67 ± 0.18^d	33.88 ± 0.35^d

Parameter	Treatment & Formulation						
	EC ₁	Defatted BSFL	K ₃	EC ₂	EC ₃	EC ₄	EC ₅
Crude Fat, %	1.10 ± 0.02 ^b	0.11 ± 0.01 ^a	0.26 ± 0.07 ^a	1.41 ± 0.06 ^c	2.02 ± 0.08 ^d	2.92 ± 0.01 ^e	3.85 ± 0.03 ^f
Crude Fibre, %	1.14 ± 0.35 ^a	19.26 ± 0.40 ^g	17.24 ± 0.25 ^f	2.94 ± 0.14 ^b	5.84 ± 0.01 ^c	11.30 ± 0.13 ^d	14.30 ± 0.37 ^e
Ash, %	4.77 ± 0.03 ^a	15.01 ± 0.20 ^e	25.47 ± 0.09 ^g	7.62 ± 0.02 ^b	10.45 ± 0.01 ^c	13.62 ± 0.08 ^d	16.28 ± 0.02 ^f
Moisture, %	28.55 ± 0.16 ^c	11.55 ± 0.42 ^b	7.94 ± 0.19 ^a	27.25 ± 0.15 ^c	28.38 ± 0.11 ^c	31.72 ± 0.37 ^d	33.47 ± 0.04 ^e

Mean within column with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$. Columns represent the mean values \pm standard error

In Table 4, the mean CP score of defatted BSFL (52.05 ± 0.31 %) was higher compared to K₃ (51.30 ± 0.26 %) in treated diet whereas the mean CP score of EC₅ was highest (33.88 ± 0.35 %) among EC₁, EC₂, EC₃ and EC₄ in formulated egg custard (23.36 ± 0.34 %, 27.88 ± 0.22 %, 30.21 ± 0.09 % and 32.67 ± 0.18 %). K₃ has lower CP compared to defatted BSFL as the addition of acidic salt help to denature protein contents of BSFL. The amount of BSFL added is directly proportional to percentage of CP content in formulated egg custards (EC₁, EC₂, EC₃, EC₄ and EC₅) as the more amount of BSFL (0 %, 25 %, 50 %, 75 % and 100 %) added, the higher the percentage of CP content in formulated egg custard. This indicates that BSFL has higher protein content compared to skimmed milk powder as protein sources for *M. rosenbergii* larvae as BSFL dry weight contain up to 50 % CP [13] compared to skimmed milk powder which only have about 34 %. In addition, 35 % to 40 % of protein with 3.2 kcal/g of gross energy level was suitable growth of *M. rosenbergii* in a clear water supply [14].

Mean percentage of crude fat score of K₃ was higher (0.26 ± 0.07 %) compared to defatted BSFL (0.11 ± 0.01 %) in treated diets whereas mean crude fat score of EC₅ was highest (3.85 ± 0.03 %) among EC₁, EC₂, EC₃ and EC₄ in formulated egg custards (1.10 ± 0.02 %, 1.41 ± 0.06 %, 2.02 ± 0.08 % and 2.92 ± 0.01 %). K₃ has higher fat content compared to defatted BSFL as salt changing the biochemical composition of BSFL in K₃. The crude fat content of formulated egg custard is increase with the reduction of skimmed milk and the addition of BSFL. This is because the source of fat is from chicken eggs (fat content: 8.7 to 11.2 per 100 g of whole egg) [15]. Protein from milk powder binding with fat from chicken eggs and protein absorb and retain fat to form emulsion with the interaction of lipids [16] after mixing. In adversely, BSFL does not form emulsion after mixing with chicken egg. According to [14], low requirement of lipid (as low as 5 %) with sufficient level of EFA (0.075 % of n-3 and n-6 HUFA) provided optimal growth of *M. rosenbergii*.

Mean crude fibre score of defatted BSFL was higher (19.26 ± 0.40 %) compared to K₃ (17.24 ± 0.25 %) in treated diets whereas mean crude fibre score of EC₅ was highest (14.30 ± 0.37 %) among EC₁, EC₂, EC₃ and EC₄ in formulated egg custards (1.14 ± 0.35 %, 2.94 ± 0.14 %, 5.84 ± 0.01 % and 11.30 ± 0.13 %). This indicates that addition of BSFL from EC₁, EC₂, EC₃, EC₄ and EC₅ (0%, 25%, 50%, 75% and 100%) has increase the crude fibre content in the feed. From the data, the highest percentage of BSFL is still within the range that *M. rosenbergii* larvae can tolerate. According to [14], *M. rosenbergii* has the ability to utilise as high as 30 % of dietary fibre. BSFL has chitin, an insoluble fibre which resulted percentage of crude fibre increase gradually against the formulated egg custard. During acidic salt treatment, salt dissolution occurs to break down fibre content [17] and might have water-soluble fibre from BSFL dissolve in acidic salt solution which making percentage of crude fibre content of K₃ is lower compared to defatted BSFL.

Based on the result obtained from Table 4, mean ash score of K₃ was higher (25.47 ± 0.09 %) compared to defatted BSFL (15.01 ± 0.20 %) in treated diets whereas mean ash score of EC₅ was highest (16.28 ± 0.02 %) among EC₁, EC₂, EC₃ and EC₄ in formulated egg custards (4.77 ± 0.03 %, 7.62 ± 0.02 %, 10.45 ± 0.01 % and 13.62 ± 0.08 %). K₃ has lower ash content compared to defatted BSFL as salt helps to break down some inorganic minerals of BSFL in K₃ during acid salt treatment. In addition, ash content of EC₁, EC₂, EC₃, EC₄ and EC₅ is directly proportional to the amount of BSFL in formulated egg custard as more amount of BSFL added, ash content is rising gradually.

From Table 4, mean moisture score of defatted BSFL was higher (11.55 ± 0.42 %) compared to K₃ (7.94 ± 0.19 %) in treated diets whereas mean moisture score of EC₅ was highest (33.47 ± 0.04 %) among EC₁, EC₂, EC₃ and EC₄ in formulated egg custards (28.55 ± 0.16 %, 27.25 ± 0.15 %, 28.38 ± 0.11 % and 31.72 ± 0.37 %). This is because K₃ was oven-dried which caused the moisture content of K₃ to reduce compared to defatted BSFL. Milk powder dissolves readily in water content [18] from chicken eggs in formulated egg custard. In adversely, BSFL does not dissolve in chicken egg liquid. Thus, the moisture content of EC₂, EC₃, EC₄ and EC₅ is increase gradually as BSFL content increasing (0 %, 25 %, 50 %, 75 % and 100 %). Based on the study from [19], utilisation on wet diet and dry diet together according to the different larval stage of *M. rosenbergii* was recommended.

3.3. Physical Properties of Experimental Feed

Colour characteristics (L^* (lightness value), a^* (red/green value), and b^* (yellow/blue value) scale) shown statistically significant ($P < 0.05$) in all treated diets and formulated egg custards. As shown in Table 5, there is no difference on the colour characteristics in defatted BSFL (L^* : 34.69 ± 0.49 , a^* : 3.93 ± 0.05 , b^* : 8.97 ± 0.06) and K₃ (L^* : 33.21 ± 0.34 , a^* : 3.68 ± 0.04 , b^* : 8.58 ± 0.04). However, the L^* scale (lightness) (59.37 ± 0.52 , 43.05 ± 0.56 , 35.80 ± 0.47 , 31.28 ± 0.54 and 29.47 ± 0.29) and b^* scale (yellowness) (28.55 ± 0.01 , 15.96 ± 0.45 , 12.45 ± 0.24 , 10.05 ± 0.12 and 8.02 ± 0.05) was decreased from EC₁, EC₂, EC₃, EC₄ and EC₅ because the percentage of BSFL added is increasing from 0 %, 25 %, 50 %, 75 % and 100 % in respectively formulated egg custard. *M. rosenbergii* larvae accepted light-coloured feed better than darker feed [13]. In addition, colour preferences of *M. rosenbergii* larvae (zoeae) were blue and white as blue-dyed egg custard shown significantly higher body weight compared to yellow colour egg custard [20].

Table 5. Colour Characteristics of Treated Diet and Formulated Egg Custard with CIE $L^* a^* b^*$ scale.

Parameter (Colourimetry)	Treatment & Formulation						
	EC ₁	Defatted BSFL	K ₃	EC ₂	EC ₃	EC ₄	EC ₅
L^* scale (lightness)	59.37 ± 0.52^c	34.69 ± 0.49^c	33.21 ± 0.34^{bc}	43.05 ± 0.56^d	35.80 ± 0.47^c	31.28 ± 0.54^{ab}	29.47 ± 0.29^a
a^* scale (redness)	-1.51 ± 0.04^a	3.93 ± 0.05^{de}	3.68 ± 0.04^{cd}	3.21 ± 0.05^b	4.12 ± 0.03^e	4.47 ± 0.08^f	3.61 ± 0.03^c
b^* scale (yellowness)	28.55 ± 0.01^e	8.97 ± 0.06^{ab}	8.58 ± 0.04^a	15.96 ± 0.45^d	12.45 ± 0.24^c	10.05 ± 0.12^b	8.02 ± 0.05^a

Mean values \pm standard error with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$.

The physical properties of treated diets and formulated egg custards were reported in Table 6. Physical properties (hardness, sinking velocity and bulk density) were statistically significant ($P < 0.05$) in all treated diets and formulated egg custard. EC₁ has the most hardness (12087 ± 0.00 g) whereas EC₅ has the least hardness (2782.50 ± 279.50 g) compared to EC₂, EC₃, EC₄ (8841.50 ± 1077.50 g, 11899.00 ± 0.00 g and 12074.00 ± 0.00 g) in formulated egg custard. This is due to the

different composition of EC₁, EC₂, EC₃, EC₄ and EC₅ depending on the amount of BSFL and skimmed milk powder added. However, hardness on defatted BSFL and K₃ is not detectable. Hardness in this study is not applicable because experimental feed requires pass-through 300 micrometre of mesh size before feeding.

Table 6. Mean Score on Physical Properties of Treated Diet and Formulated Egg Custard with Hardness, Sinking Velocity and Bulk Density.

Parameter	Treatment & Formulation						
	EC ₁	Defatted BSFL	K ₃	EC ₂	EC ₃	EC ₄	EC ₅
Hardness, (g)	12087 ± 0.00	N/A	N/A	8841.50 ± 1077.50	11899.00 ± 0.00	12074.00 ± 0.00	2782.50 ± 729.50
Sinking Velocity, (cm/sec)	0.66 ± 0.02 ^{ab}	2.37 ± 0.10 ^e	0.57 ± 0.04 ^a	0.76 ± 0.01 ^{ab}	1.00 ± 0.01 ^{cd}	1.01 ± 0.01 ^d	0.83 ± 0.02 ^{bc}
Bulk Density, (g/cm ³)	0.40 ± 0.01 ^{bc}	0.44 ± 0.01 ^d	0.42 ± 0.00 ^{cd}	0.41 ± 0.00 ^{bc}	0.39 ± 0.00 ^{ab}	0.37 ± 0.00 ^a	0.47 ± 0.01 ^e

Mean within column with different letter(s) indicate significant difference between treatments by Tukey's HSD test at $P \leq 0.05$.

As shown in Table 6, defatted BSFL has slowest sinking velocity (2.37 ± 0.10 cm/sec) compared to K₃ (0.57 ± 0.04 cm/sec) whereas EC₄ has slowest sinking velocity (1.01 ± 0.01 cm/sec) compared to EC₁, EC₂, EC₃ and EC₅ (0.66 ± 0.02 cm/sec, 0.76 ± 0.01 cm/sec, 1.00 ± 0.01 cm/sec and 0.83 ± 0.02 cm/sec) in formulated egg custards. The suitable feed of *M. rosenbergii* larvae in terms of sinking velocity were defatted BSFL and EC₄ because swimming behaviour of *M. rosenbergii* larvae are at the upper layer of water.

In Table 6, the bulk density of defatted BSFL (0.44 ± 0.01 g/cm³) has the highest bulk density compared to K₃ (0.42 ± 0.00 g/cm³). Bulk density of EC₅ has highest bulk density (0.47 ± 0.01 g/cm³) whereas EC₄ has lowest bulk density (0.37 ± 0.00 g/cm³) among others formulated egg custard, EC₁, EC₂ and EC₃ (0.40 ± 0.01 g/cm³, 0.41 ± 0.00 g/cm³, 0.39 ± 0.00 g/cm³). Based on a study from [18], high bulk density helps to reduce costs during packaging and transportation. In addition, high bulk density has more ability to fit in space of tablet or capsule [21] on feed.

4. Conclusion

The present study demonstrated that pre-treated BSFL with salt has potential be used as *M. rosenbergii* larvae feed with further improvement. Feeding trial is recommended to determine the acceptance level of *M. rosenbergii* larvae towards the improved formulated feed.

Acknowledgment

Thanks to Faculty of Agro-based Industry and Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan for supporting this research.

References

- [1] Dalsgaard J, Ekman K S, Pedersen P B and Verlhac V 2009 Effect of supplemented fungal phytase on performance and phosphorus availability by phosphorus-depleted juvenile rainbow trout (*Oncorhynchus mykiss*), and on the magnitude and composition of phosphorus waste output *Aquaculture* **286**(1) 105–112. 10.1016/j.aquaculture.2008.09.007.

- [2] Emmerson W D 1984 Predation and energetics of *Penaeus indicus* (Decapoda, Penaeidae) larvae feeding on *Brachionus plicatilis* and *Artemia nauplii*. *Aquaculture* **38**(3) 201 – 09. 10.1016/0044-8486(84)90144-3.
- [3] Giwa A, Dufour V, Al Marzooqi F, Al Kaabi M, Hasan S W 2017 Brine management methods: recent innovations and current status *Desalination* **407** 1-23. 10.1016/j.desal.2016.12.008.
- [4] Muhammad Amiruddin W, Sukri S A M, Al-Amsyar S M, Rusli N D, Mat K B, Mohd M and Harun H C 2021 Application of herbal plants in giant freshwater prawn: A review on its opportunities and limitation *IOP Conf. Ser.: Earth Environ. Sci.* **756** 012022.
- [5] Sinha R and Khare S K 2014 Protective role of salt in catalysis and maintaining structure of halophilic proteins against denaturation *Frontiers in microbiology* **5** 165 10.3389/fmicb.2014.00165.
- [6] Balqis Nur A'rsyati Binti Mohamad Noor, Nurul Najihah Binti Abdul Rojab, Muhammad, Hasnita Che Harun 2020 Feed formulation of improved egg custard formulation using response surface methodology (RSM) *IOP Conf. Ser.: Earth Environ. Sci.* **596** 012077.
- [7] Fonseca K B, Dicke M, and Van Loon J JA 2017 Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed *J. Insects Food Feed*, **3**. 105-120. 10.3920/JIFF2016.0055.
- [8] Van Huis A 2019 Insects as food and feed, a new emerging agricultural sector: a review *J. Insects as Food Feed* **6** 1-18. 10.3920/JIFF2019.0017.
- [9] Makkar H P S, Tran G, Heuzé V, and Ankers P 2014 State-of-the-art on use of insects as animal feed *Anim Feed Sci Tech* **197** 1–33. 10.1016/j.anifeedsci.2014.07.008.
- [10] Hua K, Cobcroft J M, Cole A, Condon K, Jerry D R, Mangott A, Praeger C, Vucko M J, Zeng C, Zenger K, and Strugnell J 2019 The future of aquatic protein: Implications for protein sources in aquaculture diets *One Earth* **1**(3) 316-329. 10.1016/j.oneear.2019.10.018.
- [11] Debnath P, Chowdhury S K, and Roy N C 2017 Effect of dietary slat supplementation on growth and feed utilization of tilapia (*Oreochromis niloticus*) *Int J Fish Aquat Stud* **5**(6) 275-280.
- [12] Sinha R and Khare S K 2014 Protective role of salt in catalysis and maintaining structure of halophilic proteins against denaturation *Frontiers in Microbiology* **5** 165. 10.3389/fmicb.2014.00165.
- [13] Shumo M, Osuga I M, Khamis F M, Tanga C M, Komi K K M, Subramanian S, Ekesi S, Van Huis A, and Borgemeister C 2019 The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya *Scientific Reports* **9**(1):10110. 10.1038/s41598-019-46603-z.
- [14] Mitra G, Chattopadhyay D N, and Mukhopadhyay P K 2005 Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming *Aqua Feeds: Formulation & Beyond* **2**(1) 17-9.
- [15] Réhault-Godbert S, Guyot N, and Nys Y 2019 The golden egg: Nutritional value, bioactivities, and emerging benefits for human health *Nutrients* **11**(3) 684. 10.3390/nu11030684.
- [16] Zayas J F 1997 Oil and fat binding properties of proteins. In: *Functionality of Proteins in Food* Springer, Berlin, Heidelberg. 10.1007/978-3-642-59116-7_5.
- [17] Chen J, Vongsanga K, Wang X, and Byrne N 2014 What happens during natural protein fibre dissolution in ionic liquids *Materials* **7**(9) 6158–6168. 10.3390/ma7096158.
- [18] Augustin M A and Margetts C L 2003 Powdered milk | milk powders in the marketplace. In: Caballero, B. (eds.) *Encyclopedia of Food Sciences and Nutrition (Second Edition)* 4694-4702 Academic Press.
- [19] De Barros H P and Valenti W C 2003 Food intake of *Macrobrachium rosenbergii* during larval development *Aquaculture* **216**(1-4) 165–76. 10.1016/s0044-8486(02)00505-7.
- [20] Kawamura G, Bagarinao T and Yong A 2017 Sensory systems and feeding behaviour of the giant freshwater prawn, *Macrobrachium rosenbergii*, and the marine whiteleg shrimp, *Litopenaeus vannamei* *Borneo Journal of Marine Science and Aquaculture* **1** 80-91.

- [21] Amidon G E, Meyer P J and Mudie D M 2017 Chapter 10 - Particle, powder, and compact characterization, Editor(s): Yihong Qiu, Yisheng Chen, Geoff G Z Zhang, Lawrence Yu, Rao V M Developing solid oral dosage forms (Second Edition) Academic Press, 10.1016/B978-0-12-802447-8.00010-8.