EVALUATION OF ARTIFICIAL DIET ON GROWTH DEVELOPMENT OF *Elaeidobius kamerunicus* FAUST (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

The rearing of *Elaeidobius kamerunicus* is difficult as it required the anthesis male inflorescence of oil palm as the breeding and feeding ground for the weevils. To date, utilisation artificial diets for rearing of *E. kamerunicus* has not been extensively studied. Thus, the objectives of this study were to compare growth development of the larvae using adopted artificial diet formulations and the natural rearing of *E. kamerunicus* besides determining the nutritional component of the natural food (male inflorescence of oil palm at anthesis). Three artificial diet types adopted from the diet of *Anthonomus tenebrosus* (Coleoptera: Curculionidae) and *Anthonomus grandis* (Coleoptera: Curculionidae) were compared with the natural food source towards the growth development of *E. kamerunicus*. The proximate composition of the natural food source obtained from the field was determined. The findings showed that overall mortality was significantly different for diet types evaluated ($P < 0.01$). The natural feed caused shorter life cycle of the larvae (10.85 ± 0.34 days). Sex ratio of 0.54 with higher number of female adult was also recorded on natural feed while the artificial diet caused total mortality on the larvae. Nutritional study of the male spikelet at anthesis shown it has 75% moisture content, 20% carbohydrate, 4% protein and less than 1% fat. The formulated artificial diets were found to have lesser essential nutrients to support the growth of larvae. This study provided new knowledge in the formulation of artificial diets and the importance of macronutrient composition on the growth of *E. kamerunicus*.

Keyword: *Elaeidobius kamerunicus,* artificial diet, proximate composition, growth development, sex ratio

ABSTRAK

Pemeliharaan *Elaeidobius kamerunicus* menghadapi kesukaran kerana memerlukan bunga jantan sawit yang sedang mekar sebagai sebagai tempat pembiakan dan sumber makanan kumbang ini. Sehingga kini, pengunaan diet alternatif untuk pemeliharaan *E. kamerunicus* masih tidak diselidiki secara menyeluruh. Oleh itu, objektif kajian ini adalah untuk membandingkan kemandirian hidup larva terhadap formulasi diet alternatif yang telah diadaptasi dan pemeliharaan *E. kamerunicus* secara semulajadi di samping untuk menentukan kandungan nutrisi makanan semulajadi (bunga jantan sawit). Tiga diet alternatif yang telah diadaptasi daripada diet *Anthonomus tenebrosus* (Coleoptera: Curculionidae) dan *Anthonomus grandis* (Coleoptera: Curculionidae) telah dibandingkan dengan makanan semula jadi terhadap kemandirian *E. kamerunicus.* Kandungan proksimat bagi makanan semulajadi yang diperoleh daripada ladang telah ditentukan. Dapatan kajian menunjukkan keseluruhan kematian menunjukkan perbezaan signifikan bagi diet yang dinilai ($P < 0.01$). Diet makanan semulajadi menyebabkan kitaran hidup larva yang lebih singkat pada (10.85 ± 0.34 hari). Jumlah nisbah jantina betina yang tinggi pada kadar 0.54 telah direkodkan pada diet makanan semulajadi. Bagaimanapun, didapati diet alternatif menyebabkan kematian pada kesemua larva. Kajian nutrisi terhadap spikelet jantan yang sedang mengorak didapati mengandungi 75% kelembapan, 20% karbohidrat, 4% protein dan kurang dari 1% lemak. Diet alternatif yang diformulasi mempunyai kandungan nutrisi yang rendah untuk kemandirian hidup larva. Kajian ini memberi pengetahuan baru tentang formulasi diet alternatif dan kepentingan kandungan makronutrien untuk kemandirian *E. kamerunicus.*

Kata kunci: *Elaeidobius kamerunicus,* diet alternatif, kandungan proksimat, kemandirian hidup, nisbah jantina

INTRODUCTION

Oil palm industry in Malaysia had thrived for the past four decades since the first introduction of the oil palm tree as an ornamental plant in the plantation sector. The oil palm tree plantation area in Malaysia as of December 2019 was 5.9 million hectares (MPOB 2020). Commercial plantation of oil palm tree accounted for 71.7%, while the remaining 28.3% belonged to smallholder planters. Parveez et al. (2020) reported that the Malaysian oil palm industry performance for 2019 was 19.86 million tonnes of crude palm oil (CPO), 17.19 tonnes per hectares of fresh fruit bunches (FFB), 20.21% of oil extraction rates (OER) and 16.88 million tonnes palm oil export. During the early ’70s, oil palm was believed to be wind-pollinated based on report of atmospheric pollen density by (Hardon & Turner 1967), however, Syed (1979) proved otherwise with the documentation of oil palm pollination in Cameroon by *Elaeidobius* spp. which initiated the introduction of *Elaeidobius* spp. in Malaysian oil palm estates.

Elaeidobius kamerunicus (Coleoptera: Curculionidae) is the main oil palm pollinator in Malaysia. The weevil was introduced in Malaysia from Cameroon in the early ’80s to alleviate the problem faced in Malaysian oil palm estates which were a high cost for assisted pollination and low fruit production (Syed 1981; Syed et al. 1982). The selection of *E. kamerunicus* was based on its biological characteristic that was suitable for the tropical climate. *Elaeidobius kamerunicus* was reported to have a high percentage of pollen load (Dhileepan 1992; Kouakou et al. 2014; Syed 1980) with a greater percentage of germinated pollen grain (Kouakou et al. 2014), the high population during wet and dry climates (Syed 1981), moderate searching ability (Syed 1981) and host-specific to *Elaeis* sp. particularly male inflorescence of oil palm (*E. guineensis)* (Adaigbe et al. 2014; Karim 1982; Syed 1981) with lesser population abundance on *E. oleifera* variety (Melendez & Ponce 2016).

The *E. kamerunicus* was first introduced in Peninsular Malaysia followed by the Borneo region in 1981 (Syed et al. 1982). A significant attribute from the introduction of this weevil in Malaysia was increased fruit set at 15 to 30% in the Peninsular Malaysia region while in the Borneo area, the fruits set was similar to assisted pollination (Syed et al. 1982). Besides, Wahid et al. (1983) reported an increase by 41 to 54% of fruit set in 333 estates planted with Dura and Tenera oil palm breeds which showed the productive role of *E. kamerunicus* brought to the Southeast Asia region (Syed et al. 1982) especially Malaysia and Indonesia that are known as the main producers of oil palm in the world (Sawe 2018).

Nevertheless, despite the significant importance of *E. kamerunicus* in Malaysian oil palm industry, there is lack of information regarding the performance, biology and adaptation of the weevil towards Malaysia climate that could be attributed to the difficulty of rearing the weevils within a closed facility such as laboratory due to the need for anthesising male inflorescence (AMI). For instance rearing of *E. kamerunicus* was conducted by (Girsang et al. 2017; Tuo et al. 2011) utilising the anthesis male inflorescence. The anthesising male inflorescence (AMI) of oil palm is the only food source to support the life development, breeding and feeding of the whole stage of *E. kamerunicus* (Syed 1981; Zulkefli et al. 2020) where male inflorescence of oil palm production is known to be influenced by climate. Research related to *E. kamerunicus* often requires routine AMI to sustain the weevils especially those reared in the laboratory reported (Girsang et al. 2017; Tuo et al. 2011). The scarcity to obtain the AMI routinely is one of the research limitations which led to the potential development of an artificial diet to support weevil population survival during the research period.

Therefore, this study aimed to develop an artificial diet to rear *E. kamerunicus* based on the adopted diet of *Anthonomus* sp. and determine the nutritional properties of natural feed (AMI) to sustain the growth development of *E. kamerunicus. Anthonomus* sp. is a plant-feeding insect where *A. grandis* (Coleoptera: Curculionidae) is a pest that feeds on cotton plants (Jeger et al. 2017) while *A. tenebrosus* (Coleoptera: Curculionidae) hosted several *Solanum* plants for an instance, tropical soda apple (weed species) as a potential biological control agent for invasive weed program (Medal et al. 2011). *Anthonomus* sp. diet was selected as the weevil possesses similar characteristics with *E. kamerunicus* in term of body size, in the weevil family (Curculionidae) and feeding regime of plant tissue.

MATERIALS AND METHODS

Sampling Location

The anthesising male inflorescence (AMI), larvae of *E. kamerunicus* and spikelet free from fungal infection were collected from an oil palm cultivation area of University Agriculture Park (Universiti Putra Malaysia) at GPS (N2° 59' 18.7008" E101° 43' 23.16") respectively. The oil palm area was located beside the Banquet Hall UPM. The oil palm breed grown in the field was Dura x Pisifera. The oil palm was planted in May 2012 with a total planted area of four hectares and the planting distance of the cultivation area was 8.8 m x 8.8 m. This site was selected as the sampling plot as the height of oil palm was in the range of 3 m to 5 m and convenient for routine bagging and collection of male inflorescences. The selection was based on a report by Tan et al. (2014), where oil palms of less than 10 years were estimated to have an average height of less than 4 m with a positive correlation between oil palm age and height of palm noted.

Elaeidobius kamerunicus **Stock Culture Preparation**

The stock culture of 3rd instar larvae *E. kamerunicus* was established by first the male inflorescence of oil palm before anthesis was bagged tightly to prevent infestation by other insects. When the male inflorescence reached full anthesis where the floret opened and the presence of yellowish pollen surrounding the spikelet was observed, the bag was removed. This allowed the wild adult *E. kamerunicus* to undergo mating and oviposit the egg inside the floret. The mating period was between 24 to 48 hours. The method used was modified from Girsang et al. (2017) and Hussein and Rahman (1991). The spikelet was cut post 48 hours mating and brought to the laboratory to collect the larvae by viewing under a dissecting microscope.

Preparation of Artificial Diet

The ingredients used for the development of artificial diet were agar (Sigma), sucrose (Sigma), Vanderzant vitamin mixture for insects (Sigma-Aldrich), myo-inositol (Sigma), choline chloride (Sigma), Wesson salt mixture (MP Biomedicals), soy protein isolate (MP Biomedicals), cholesterol (Sigma), L-ascorbic acid (MP Biomedicals), potassium sorbate (Sigma-Aldrich), linseed oil (Sigma-Aldrich), soybean oil (Sigma), L-proline (MP Biomedicals) and 95% ethanol (Hamburg). The ingredient following (Davis 2007).

The formulation of artificial diet is shown in Table 1. Diet 1 was adopted from a diet designed for *Anthonomus grandis* (Earle et al. 1966), while, Diet 2 and Diet 3 were adopted diets for *Anthonomus tenebrosus* (Davis 2007) with a slight modification of the composition of the main ingredients. All the artificial diets were compared with natural feed which was the fresh anthesising male inflorescence (AMI) of oil palm free from insect and fungal infestations. The AMI was transversely cut into smaller sections of 4 cm long which was placed in a rearing cup to feed the larvae of *E. kamerunicus.*

Table 1. Composition of three types of artificial diet formulation

Ingredients	**Weight**		
	Diet 1	**Diet 2**	**Diet 3**
Agar	3 g	3 g	3 g
Water	160 ml	160 ml	160 ml
Sucrose	8 g	8 g	8 g
Vanderzant vitamins	0.02 g	0.02 g	0.02 g
Inositol	0.04 g	0.04 g	0.04 g
Choline chloride	0.10 g	0.10 g	0.10 g
Wesson salt mixture	1.34 g	1.34 g	1.34 g
Soy protein isolate	5 g	5 g	5 g
Cholesterol	0.10 g	0.10 g	0.10 g

L-ascorbic acid	0.40 g	0.40 g	0.40 g
Male oil palm spikelet	0 g	0.18 g	0.18 g
Lipids	0 ml	0 ml	1.28 ml
L-proline	0 g	0 g	2 g
Inhibitor mixture	2.50 ml	2.50 ml	2.50 ml

Note: Diet 1: Earle et al. 1966; Diet 2 and Diet 3: Davis 2007

The preparation of artificial diet was carried following the method described by Davis (2007). The agar was dissolved in distilled water and the mixture was boiled for a minute. After that, the mixture was cooled until 75 °C before other ingredients were added. Inhibitor mixture was prepared using 10 g of potassium sorbate and 100 ml of 95% ethyl alcohol with a ratio of 1:10. The inhibitor mixture was added to the diet formulation at a concentration of 1.25 ml/ 100 g of diet. The inhibitor mixture was added to preserve the diet from mold, yeast, fungal and bacterial infection. Finally, 10 ml of the diet mixture was poured into a moulding cup and fed to the *E. kamerunicus* larvae. Every three days once, the diet was replaced with a new one to prevent mould and fungal development.

The artificial diet (Diet 2 and Diet 3) was developed using a similar method as the other diet constructed with the addition of AMI of oil palm. The preparation of the AMI was the first following procedure of bagging of male inflorescence until achieved anthesis before collected. Then, the spikelet was cut individually before stored in a deep freezer (-20 to-80°C). The spikelet was later sent to the freeze dryer machine to remove the spikelet's moisture and water. The dried spikelet was then ground into small particles and was stored in airtight screwcap bottle prior to use.

E. kamerunicus Growth Development Monitoring Under Artificial Diet

For each cup of artificial diet and natural feed, 12 individuals of 3rd instar larvae were selected and the cumulative weight of each group of larvae according to assigned diet was obtained with total of 60 samples utilised in each diet. A total of five group was created for each diet. A daily record of mortality and growth development of the larvae *E. kamerunicus* was monitored until the larvae achieved adulthood stage. The sex ratio was calculated using the following formula outlined by Campos et al. (2017) and Butt & Cantu (1962) as follows:

$$Sex\ ratio = \frac{Total\ number\ of\ female\ adult}{Total\ number\ of\ adult}$$

The rearing temperature of 26 °C to 30 °C and relative humidity of 60% to 80% were used throughout the growth development of larvae *E. kamerunicus*. Every three day the diet and the natural feed was replaced with new diet by transferring the larvae *E. kamerunicus*.

Proximate Analysis of at Anthesis Male Inflorescence of Oil Palm

The sample of AMI of oil palm was prepared where pre-anthesis male inflorescence was first bagged to prevent from infestation by other insects in the oil palm field. When the male inflorescence achieved full anthesis (presence of yellowish pollen and floret was fully opened), the spikelet was collected and stored in a freezer at -20 °C before subjected to proximate analysis.

The proximate composition (moisture, total fat. ash content, protein, carbohydrate) and energy of AMI from oil palm were analysed following AOAC (2012) standard methods. The selection of AMI of oil palm due to its function as the breeding and feeding sites for the *E. kamerunicus.* Thus, greater reference is needed to identify the nutritional value of the AMI.

Determination of Protein

The Kjeldahl method was used to determine the percentage of protein in the sample using titration with acid method following AOAC 981.10 method (AOAC 2012) and the protein content was calculated using the formula as follows:

$$\%\ \text{Protein} = \frac{(\text{ml sample} - \text{ ml blank}) \text{x HCl conc x } 1.4007 \text{ x factor}}{\text{sample weight (g)}}$$

where protein factor = 6.25

Determination of Moisture Content

Moisture content was measured using the oven drying method following AOAC 950.46 method (AOAC 2012). A well ground sample was dried in an oven until constant weight. The difference between the initial weight and constant weight of the sample after drying were recorded and the following formula was used for calculation:

$$\%\ \text{moisture} = \frac{(\text{W1} - \text{ W2})\text{ x } 100\%}{\text{Ws}}$$

where

W1= weight of sample and dish before drying.

W2= weight of sample and dish after drying.

Ws= weight of the sample

Determination of Total Fat

The total fat of sample was analysed and calculated following AOAC 991.36 method (AOAC 2012). The determination was using the hydrolysis method with strong acid. The extracted oil was calculated as follows:

$$\%\ \text{Fat} = \frac{(B - A)\ \times 100}{C}$$

where

A = weight of extraction cup before extraction, in g

B = weight of extraction cup after drying, in g

C = sample weight

Determination of Total Ash

The total ash content was determined by combustion of organic matter using muffle furnace at 550 ^{0}C to constant mass following AOAC 923.03 method (AOAC 2012). The following formula was used to calculate total ash:

$$\%\text{Total ash} = \frac{(\text{W1} - \text{W2})\ \times 100}{\text{Ws}}$$

Where,
W1 = Weight of ash and ashing dish
W2 = Weight of ashing dish
Ws = Weight of sample

Determination of Total Carbohydrate

The carbohydrate content was calculated by difference according to the formula outlined by Pomeranz and Meloan (2000) as follows:

$$\% \text{ Carbohydrates} = 100 - (\% \text{ Protein} + \% \text{ Fat} + \% \text{ Ash} + \% \text{ Moisture})$$

Calculation of Total Energy

Conversion factors as recommended for Atwater energy calculation based on Pearson (1976) were used as follows:

$$\text{Energy (kcal)} = 4(\%\text{Carbohydrate}) + 4(\% \text{ Protein}) + 9(\%\text{Fat})$$
$$\text{Energy (kJ)} = \text{Energy (kcal)} \times 4.2$$

Statistical Analysis

The growth development parameters recorded for *E. kamerunicus* were weight gain, mortality, sex ratio and life development (egg to adult). The experimental design implemented was a completely randomised design with N= 60 represent the replication of each diet. Group weight gain measurement of 12 individual 3rd instar larvae was recorded and mean value of the group was express as individual weight of the survived larvae according to group. It was then analysed using one-way Analysis of Variance (ANOVA). Followed up by mean comparison using LSD at 5%. The overall mortality data were analysed using the Kruskal-Wallis test and comparison was carried out using the Dwass, Steel, Critchlow-Fligner pairwise comparison method. The interaction between diet and diet exposure hour towards daily mortality was analysed using Analysis of Variance (ANOVA). Whereas the sex ratio was analysed using the chi-square test of goodness of fit with the standard of sex ratio from research by Herlinda et al. (2006).

The proximate analysis result was compared with the main constituent used in the artificial diet of Davis (2007) and Earle et al. (1966) and low cost diet of Sarah Najihah et al. (2019) to determine the nutritional value through calculation of nutritional value obtained in each diet (g/100g). All the data were statistically analysed using the SAS 9.4 version software.

RESULT

Growth Development of *E. kamerunicus* as Affected by Adopted Diet

The weight gained by one-week old *E. kamerunicus* larvae was significantly different between the types of diets tested ($F= 39.51$, $P < 0.01$). As shown in Figure 1, larvae feeding on artificial diet (Diet 2) resulted in the highest weight gain in contrast to the lowest weight gain of larvae observed when the naturally fed.

Although the larvae gained weight with artificial diet, most of the larvae fed with artificial diets were affected by high mortality. The mortality rates of larvae were determined to understand the larval response after being fed with artificial diet.

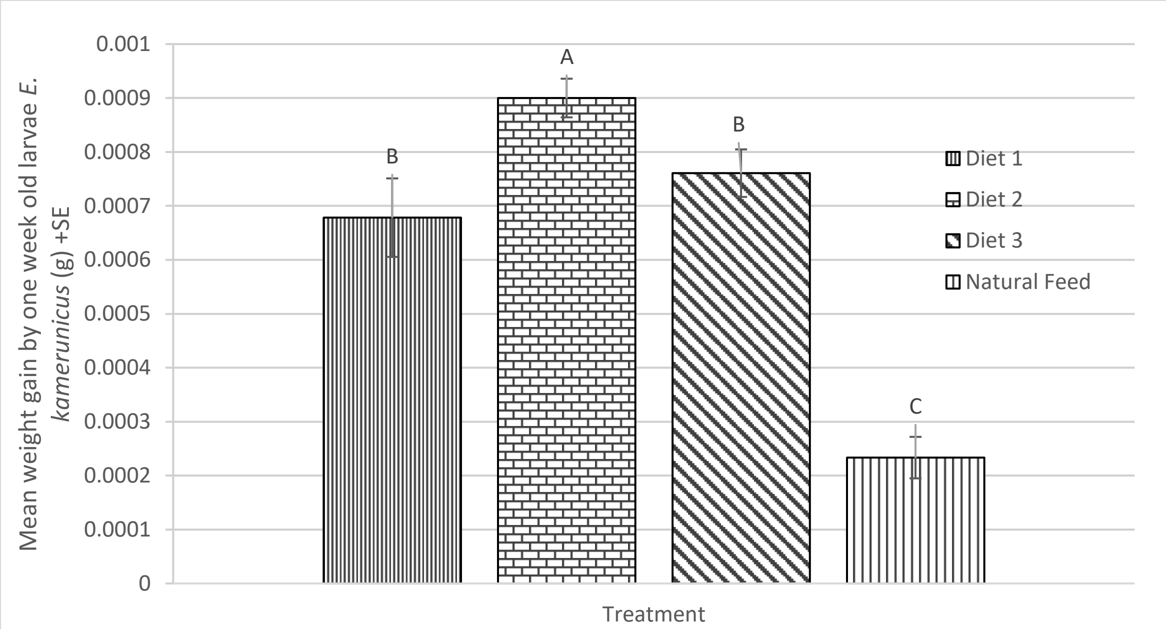

Figure 1. The effect of diet types on the mean weight gain of one-week-old larvae *E. kamerunicus* +SE. Means with similar letter are not significantly different at P > 0.05 using LSD

The Mortality Rate of *E. kamerunicus* Larvae Fed on Adopted Diets

No significant interaction between diet types and diet exposure hours (F= 0.83, P > 0.05, df= 24) on the mortality rate of *E. kamerunicus* larvae were noted. The mortality of *E. kamerunicus* larvae increased with an increase of diet exposure hours for all diet types evaluated including the natural feed (Figure 2). Nevertheless, the natural feed does not cause larvae mortality rate to be greater than 80% at the final assessment while the artificial diet led to the highest larvae mortality of 100% at the end of the assessment. The Kruskal-Wallis test result showed that treatments were significantly different at (Chi-square= 41.0617, P < 0.01, df=3) for the overall mortality. The pairwise comparison test finding indicated that the natural feed was significantly different from the artificial diets (Diet 1, Diet 2 and Diet 3) at P < 0.01 in affecting the mortality rate of larvae (Table 2) suggesting natural feed to be superior to the artificial diets formulated in this study.

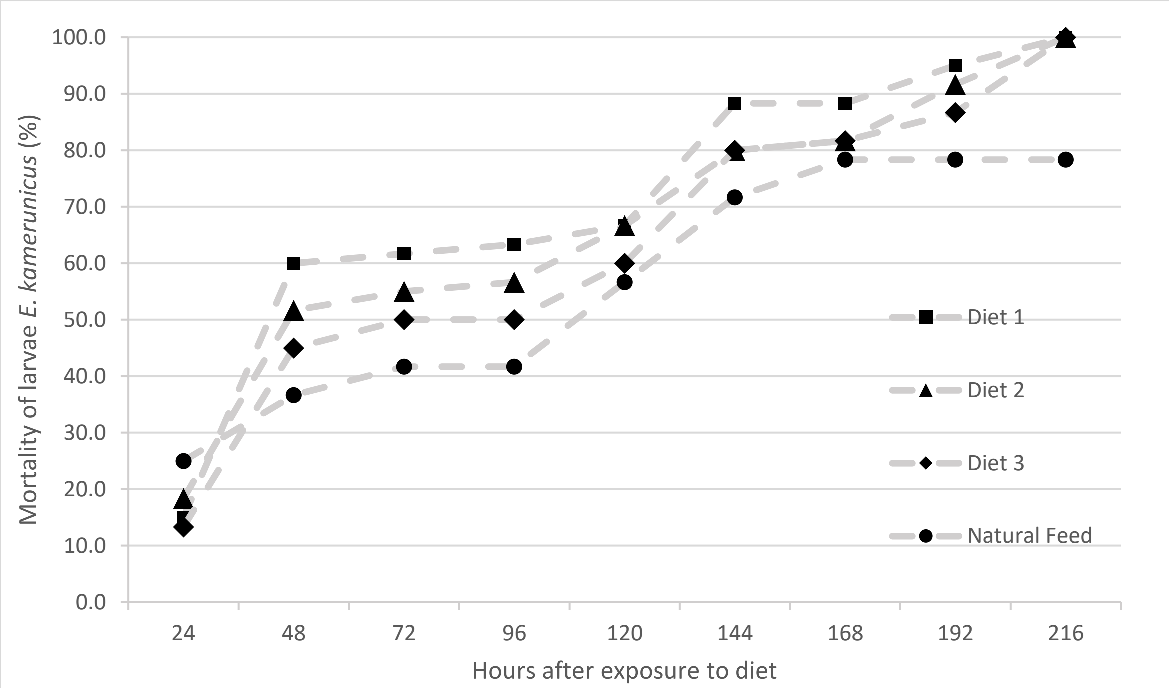

Figure 2. The mortality (%) of *E. kamerunicus* larvae affected by hours of exposure to diet

Table 2. Pairwise comparison of diet types effect on the overall mortality (%)

Treatment	**Significant value**
Diet 1 vs. Natural Feed	0.0008*
Diet 2 vs. Natural Feed	0.0008*
Diet 3 vs. Natural Feed	0.0008*

*Highly significant at P < 0.01 using Dwass, Steel, Critchlow-Fligner pairwise comparison method

Sex Ratio and Life Development of *E. Kamerunicus* Larvae Fed with Natural Feed

Sex ratio and life development of *E. kamerunicus* larvae fed with only natural feed were recorded as the other treatments caused 100% mortality of larvae at the end of the assessment. The sex ratio of the larvae fed with natural feed was 0.54 (female= 7, male= 6) with a higher number of female *E. kamerunicus*. In previous report by Herlinda et al. (2006) recorded adult *E. kamerunicus* emergence of 53.6% female as compare to the male weevil. The sex ratio was evaluated as standard reference with the natural feed adult emergence. The analysis tabulated Chi-square= 0.0003, df= 1 and P > 0.05. This indicates insignificant difference with the standard. In the experiment it was observed the life development recorded was 10.85 ± 0.34 days from egg to adult *E. kamerunicus* for the natural feed.

Macronutrient Values of at Anthesis Male Inflorescence of Oil Palm

The artificial diets formulated caused total mortality of larvae at the end of the assessment that could be due to lack of nutrients needed for growth development of *E. kamerunicus* larvae as compared to natural feed diet (at anthesis male inflorescence of oil palm).

The at anthesis male inflorescence of oil palm (Table 3) spikelet predominantly consisted of moisture (75%) and carbohydrate (20%) followed by protein, ash and fat which are essential to support the growth of *E. kamerunicus.* The proximate composition of at anthesis male inflorescence of oil palm indicated that carbohydrate was predominant macronutrient found. The adopted artificial diets did not support the growth development of *E. kamerunicus* larvae possibly due to lack of macronutrient content in comparison to natural feed. The artificial diet was formulated with 5 g of soy protein isolate which contributed 60% of protein and 20% of total fat whereas 8 g of sucrose and 3 g of agar contributed 30% of total carbohydrate based on g/100g compared to the natural feed as shown in Table 3 which indicated lack of essential nutrient to support the growth of weevils.

Table 3. Proximate composition of the natural feed for *E. kamerunicus* compared to the conventional protein used in the development of artificial diet and major constituents of low-cost diet for *E. kamerunicus*

Parameter/ Unit	**Anthesising male inflorescence (AMI) of oil palm**	**Main ingredient in *E. kamerunicus* diet formulation and Low-cost diet (Sarah Najihah et al. 2019)**					
		Soy protein isolate	**Wheat flour**	**Corn flour**	**Yeast**	**Milk powder**	**Honey**
Protein, g/100g	4.1	88.32	11.8	10.34	40.44	45.71	
Total fat, g/100g	0.4	3.39	1.1	5.17	7.61	17.14	
Total Carbohydrate, g/100g	20.3		70.7	75.86	41.22	18.5	80.95
Ash, g/100g	1.9	3.58				3.65	
Moisture, g/100g	73.3	4.98				15	
Energy, kcal/100g	101 (424kJ)	335	345	379	325	411	286
Reference		(USDA 2019)	(USDA 2020d)	(USDA 2020a)	(USDA 2020e)	(USDA 2020c)	(USDA 2020b)

DISCUSSION

Dried spikelet used in the development of an artificial diet were shown to act as potential phagostimulant for larvae feeding. In this study, *E. kamerunicus* larvae fed with artificial diet 2 with the presence of dried spikelet resulted in the highest weight gain by the larvae which was greater than the natural feed. In a previous study, *Anthonomus grandis* feeding was stimulated with the presence of secondary compounds such as gossypol in cotton plants (Nation 2016). Earlier, Ma (1972) stated that sinigrin compound in mustard plant stimulated the feeding of *Pieris brassicea*, a cabbage butterfly. Sinigrin compounds were reported to stimulate oviposition mechanism by the cabbage butterfly even in harsh conditions (Nation 2016). According to Hussein and Lajis (1992) and Syed (1981), both male and female inflorescences of oil palm were able to produce a volatile compound called estragole to attract pollination by *E. kamerunicus*. In the summary by Zulkefli et al. (2020) it was documented the estragole present in the pollen (Opute 1975) and both inflorescence of oil palm (Lajis et al. 1985; Muhamad Fahmi et al. 2016) with addition of soil cultivated area have effect on the estragole production (Muhamad Fahmi et al. 2016). Nevertheless, the study of estragole compound in stimulating the feeding of *E. kamerunicus* is not available.

Mortality of larvae is the main concern during the research of artificial diet impact on growth development of larvae reared in a closed facility where mortality of 60% was noted for *E. kamerunicus* larvae in a study conducted in Malaysia (Hussein & Rahman 1991). This could be due to the disruption of larvae during the development process as a part of the experiment such as tearing of spikelet to observe the growth development and transferring of the larvae to new spikelets to prevent fungal infection. This was further confirmed where, when no disruption occurred during the study of *E. kamerunicus* larvae performed in a laboratory, no mortality was observed (Girsang et al. 2017; Tuo et al. 2011). In contrast, when larvae were disrupted during rearing for instance transferring the survived larvae to new diet, 78% of overall mortality was noted larvae fed with natural feed while total mortality was reported for larvae fed with artificial diet. Despite the development and feeding of *E. kamerunicus* was influenced by male inflorescence of oil palm (Zulkefli et al. 2020), the mortality on the natural feed was believe to be influence by the rearing procedure during this experiment where every three days changes of diet to prevent fungal infection thus disrupting the larvae development. Whereas the artificial diet mortality was believed due to low nutritional value of the artificial diet.

Besides, in this study, a sex ratio which was susceptible to female adult was noted. A similar finding was reported by Herlinda et al. (2006) where a sex ratio of 0.536 with a higher percentage of female was noted when *E. kamerunicus* larvae were reared inside a laboratory in Indonesia. Rearing larvae in a closed environment-induced formation of a female adult, however, the reasons remain unclear. The finding similar to report of sampling in field where highest number of female adult *E. kamerunicus* on male inflorescence (Rahardjo et al. 2018; Rizali et al. 2019) and also during anthesis period of male inflorescence of oil palm (Yue et al. 2015). The life cycle from egg to adult for the larvae was reported to vary according to region. The pioneering research conducted by Syed (1981) in Cameroon, the native country of *E. kamerunicus* reported a life cycle of 19 days. Research in Malaysia conducted a year later recorded a life cycle between 7 to 14 days during quarantine before introduction to oil palm plantations in Malaysia (Karim 1982) and in another report, a life cycle of 15 days when reared inside laboratory (Hussein & Rahman 1991). Meanwhile, egg to the adult life cycle of *E. kamerunicus* in other countries such as the Ivory Coast was 15 days (Mariau et al. 1991) and 14 days (Tuo et al. 2011) and in a similar climate life cycle of 14 to 16 days (Herlinda et al.

2006) and a shorter period of 12 days (Girsang et al. 2017) were reported in research conducted in Indonesia.

In the present study, the growth development of *E. kamerunicus* from immature stage to adult was 10.85±0.34 days which was shorter than that reported in the previous studies. This could be induced by higher temperature as suggested by Herlinda et al. (2006). Cooper and Cave (2016) reported that the development from egg to adult of a bromeliad feeding weevil, *Metamasius callizona* (Coleoptera: Curculionidae) was influenced by temperature during rearing. Temperature more than 25 °C reduced the bromeliad feeding weevil development from egg to adult while temperature between 28 to 30°C reduced the pupal development period and the weevil development was halted when the temperature was greater than 33°C. In this study, *E. kamerunicus* larvae were reared at an average temperature of 28.8 °C ± 0.27, slightly higher than 25 °C that could induce faster development from egg to adult in 11 days.

Sarah Najihah et al. (2019) reported the successful rearing of *E. kamerunicus* larvae using a low-cost diet with 56% of adult emergence. The development period from egg to adult was between 10 to 11 days. This report indicated that the artificial diet formulated by Sarah Najihah et al. (2019) was optimised for the rearing of *E. kamerunicus* but performance comparison with the natural feed was not carried out with only macronutrient values of the main constituents of the low-cost diet was quantified as presented in Table 3. The low-cost diet utilised 192 g of wheat flour, 80 g of corn flour, 48 g of milk powder, 263 g honey and 7 g yeast which supplied 6.2 g/100g of protein, 1.68 g/100g of total fat and 46.9 g/100g of total carbohydrate when compared with the natural feed (Table 3) where the macronutrient content was within 1.5 to 4 times higher (Sarah Najihah et al. 2019) which indicated the diet formulated contain all the essential macronutrients required to support the growth of *E. kamerunicus.*

The population of *E. kamerunicus* is essential for effective pollination in oil palm plantation in Malaysia. In the summary by Zulkefli et al. (2020) documented several factor effecting the population of *E. kamerunicus* for instance other insect preying on the weevil (Muhammad Luqman et al. 2017), synthetic pesticide (Yusdayati & Hamid 2015) and climate factor (Nurul Fatihah et al. 2018). With the effective method to mitigate the population of *E. kamerunicus* through hatch and carry technique by (Prasetyo et al. 2014). Thus, the application of artificial diet for rearing of the *E. kamerunicus* will facilitate the main problem of rearing the weevil through substituting the need for the natural feed. The current prospect of rearing *E. kamerunicus* in laboratory environment using low cost diet by (Sarah Najihah et al. 2019). Further research is needed to develop the best diet for rearing of the *E. kamerunicus.*

The deficiency of macronutrient generally affects the growth development of insects. Insects are similar to other vertebrates where 10 essential amino acids for growth are required (Cohen 2015; Nation 2016). Protein is needed for an insect to obtain nitrogen and used for the development of muscle and cell membrane, and production of enzymes and hormones (Cohen 2015). Insufficient protein supply failed to produce juvenile hormone needed for the reproductive system in female insects whereas none reported for male insects (Nation 2016). Genc (2006) reported that *Pectinophora gossypiella, Helicoverpa zea, Myzuz persicae, Tribolium confusum* and *Apis mellifera* development were incomplete in the absence of those 10 essential amino acids namely arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, trythophan, and valine. In a report by Lee (2007), the cotton leafworm, *Spodoptera littoralis* fed with a diet containing low quality of protein caused reduction of body weight and slow development of the caterpillar.

Besides protein, carbohydrate is an essential nutrient as an energy source for insects (Cohen 2015; Nation 2016) and as a building material (Cohen 2015). Several species of insects require carbohydrate to achieve maturities such as *Tenebrio* sp., *Ephestia* sp. and *Oryzaephilus* sp. while there exist some insects which can also reach adulthood in a low carbohydrate diet (Nation 2016). In a study conducted by Filho et al. (2018), the biological development of sugarcane borer, *Diatraea saccharalis* was influenced by the concentration of sugar used in the diet provided during the experiment. They also observed that sugar concentration between 26.26 and 52.52 g per litre of diet influenced the survivability of *D. saccharalis* development from egg to adult with the viability of more than 75% for eight generations tested. The moderate sugar concentration also influenced shorter generation time and greater reproduction rate of the sugarcane borers as compared to other sugar concentrations tested.

Lipid is also known to be important for the growth of insects especially for cell membrane development, hormone production, nutrient transportation, as a fuel and building material for other molecules. Sterol is among the specific lipids required by insects as they were unable to synthesise and have to obtain sterol in the diet (Cohen 2015; Nation 2016). Sterol is essential for ecdysteroid moulting hormone production, a component in the cell membrane (Nation 2016) and aid in transporting of materials inside the cell (Cohen 2015). Nation (2016) stated that sterol deficiency resulted in the inability of newly hatch larvae to survive the immature stage due to fully utilised the sterol obtained from mother during hatching. Besides, scolitid beetle (*Xyleborus ferrugineus*) also failed to pupate as the presence of sterol is needed for moulting hormone synthesis.

CONCLUSIONS

The adoption of diet for *Anthonomus* sp. was documented unsuitable for rearing the immature stage of *E. kamerunicus* due to the formulated diet lacked in essential nutrients as compared to the natural feed (anthesising male inflorescence) with only 60% protein, 20% total fat and 22% of the total carbohydrate. The use of natural feed caused faster growth to immature weevil with a life cycle of 10 days and produced adults with a high percentage of females. The mortality of the reared *E. kamerunicus* larvae ranged from 78% to 100% depending on the feed provided. The findings from this research suggest the potential use of an artificial diet to increase the weight gain of *E. kamerunicus* larvae. However, to achieve successful adult emergence of the *E. kamerunicus*, the artificial diet formulation needs to be optimised where sufficient nutrients should be supplied.

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