PROXIMATE NUTRIENT COMPOSITION OF Chrysomya megacephala AND Chrysomya rufifacies REARED ON BEEF SUBSTRATES

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Abstract

Fishmeal remains the primary ingredient in animal feeds. Its decreasing availability in natural resources coupled with the lack of several essential amino acids in plant proteins have triggered the needs for exploring alternatives avenues for protein resources. The present research investigated the proximate nutrient compositions in *Chrysomya megacephala* and *Chrysomya rufifacies* that fed on beef muscle (n=5) and liver (n=5) substrates decomposing in a sunlit habitat within UniversitiTeknologi Malaysia, Johor. Using an in-house method modified from the Association of Official Analytical Communities (AOAC) (2000), the composition of crude protein, crude lipids, carbohydrate, crude fibre, ash and moisture were analysed. It was found that the third instar larvae of *C. megacephala* and *C. rufifacies* reared on both types of beef substrates contained substantial amounts of crude protein, crude lipids, carbohydrate and crude fibre; at least comparable to that of *H. illucens*.It was observed that the use of beef muscle as rearing substrates facilitated the attainment of maximum composition of crude protein and crude lipids in the third instar larvae of these two species. On the other hand, the use of beef liver substrates has resulted in the optimum compositions of carbohydrate and crude fibre. Hence, the findings reported here support the application of these two prevalently found necrophagous species as the alternative nutritional resources for animal feeds.

Keywords: proximate nutrient compositions, Chrysomya megacephala andChrysomya rufifacies, beef muscle and liver substrates, animal feeds

INTRODUCTION

Despite the limited availability of natural resources, a substantial increase by as much as 60-70% in the consumption of animal products is expected to occur by year 2050, demanding for enormous supply of animal feeds (Makkar et al., 2014). It has been reported that the livestock production occupies about 75% of agricultural land areas and consumes about 8% of water resources for irrigation of feed crops (Foley et al., 2011). Studies have indicated that fishmeal is the primary protein source for the formulation of animal feeds in aquaculture (Li et al., 2009), poultry as well as swine farming (Hardy and Tacon, 2002) due to its high crude protein content (about 50%) and essential amino acids (Marley, 1998).

Although the use of soybean meal have been suggested, plant proteins contain inadequate amounts of cysteine, methionine and taurine (Kerr and Kidd, 1999; Brinker and Reiter, 2010) as well as severalantinutrient substances e.g. trypsin inhibitor, haemagglutinin and antivitamins (Tacon, 1993), its applicability for animal growth remains limited. This situation has increased the demand for fishmeal (Tacon and Metian, 2008; Ayoola, 2010) from the endangered pelagic fishes and may become a possible detrimental threat to marine ecosystems. Since food sources from marine environment such as fishes and prawns form considerable proportion of human diets (Duarte et al., 2009), interruption to the marine ecosystem may lead to disturbance in the food supply, causing huge competition between human needs and livestock (Makkar et al., 2014). Therefore, such a stiff competition for food ingredients for fulfilling the needs of both humans and livestock has resulted in escalating production costs that led to the search for alternative resources (Barroso et al., 2014). In this context, insect rearing may prove as one of the better ways for sustaining feed security (Newton et al., 2005; Kroeckel et al., 2012; Rumpold et al., 2013; van Huis et al., 2013; Ossey et al., 2014).

It has been reported that insects form a part of human diet especially in Asia, Latin America and Africa (Bukkens, 1997), supplementing the nutrition for approximately two billion people (Makkar et al., 2014). In view of utilizing insects in livestock farming, studies have been focusing mainly on the possibility of using the larvae and pupae of *Hermetiaillucens* (soldier flies) as the candidate in animal feed formulation due to its nutritional values and availability in large quantity (Odesanya et al., 2011). However, the fact that *H. illucens* requires several weeks to months for completing its life cycle (Veldkamp et al., 2012), longer durations for maintaining the colony prior to harvesting are required. In addition, the rearing process of the larvae of *H. illucens* may attract public criticisms with regards to its cleanliness since those larvaeare commonly reared on pig manure (Newton et al., 2005) and poultry manure/droppings (Sheppard et al., 1994; Odesanya et al., 2011), which may be disgusting to some quarters of community. Hence, utilization of other insect species with shorter durations for completing life cycles infesting more favourable substrates such as beef muscle and liver deserves considerations.

It is pertinent to indicate here that other dipterans such as *Chrysomyamegacephala* (Fabricius) and *Chrysomyarufifacies* (Macquart) are prevalently found in Malaysia (Lee et al., 2004). Being a necrophagous species, infestation by *C. megacephala* in decomposing carcasses/corpses has been reported to precede that of

C. rufifacies (Mahat et al., 2009). Since the completion of life cycles for *C. megacephala* and *C. rufifacies* has been reported to take place in 7.25 and 9 days respectively (Lee, 1989), the use of these species may provide feasible alternatives to that of *H. illucens*.Apart from the two recent studies that reported about the proximate nutrient compositions in *C. megacephala* larvae and pupae(Barroso et al., 2014; Sing et al., 2014), review of the literature reveals no specific studies focusing on exploring the comprehensive essential nutritional contents in such species wild-rearing on the different types of beef substrates. Despite reporting the proximate nutrient composition in the larvae of *C. megacephala*, no indication is provided by these researchers (Barroso et al., 2014; Sing et al., 2014) on the larval instar stages as well as time elapsed between the onset of pupation and the time of analysis for such specimen, rendering difficulties in making the appropriate comparisons. Furthermore, similar proximate nutrient data for *C. rufifacies* remain unreported.Therefore, considering that *C. megacephala* and *C. rufifacies* are ubiquitous throughout Malaysia and they too may contain the required nutritional elements similar to that of *Hermetiaillucens*, specific studies investigating the potential of those necrophagous insects as possible candidates for animal feed formulation deserve special consideration.

This present research attempted to provide empirical data on the proximate nutrient compositions in two prevalent necrophagous species in Malaysia (i.e. *C. megacephala* and *C. rufifacies*) reared in two types of continuously decomposing beef substrates under known field conditions. In addition, this present research also provided the data on proximate composition of nutrients in the third instar larvae of *C. rufifacies*, elucidating, for the first time, the potential of this species for animal feeds. The suitability of utilizing beef muscle and beef liver as rearing substrates for *C. megacephala* and *C. rufifacies* was also investigated in this present research.

EXPERIMENTAL

For studying the proximate nutrient compositions of *C. megacephala* and *C. rufifacies,* the fully grown third instar larvae of these two species reared on decomposing beef muscle and liver substrates within the Universiti Teknologi Malaysia (UTM) Johor Bahru Campus were collected during January-February 2015. The two types of substrates purchased from a nearby market, weighing about 1 Kg each, were chopped into smaller portions prior transporting them in separate sealed plastic bags to the decomposition site. A rectangular plastic container (18cm x 12cm x 6cm) was used for containing each substrate (about 1 Kg), while for preventing water retention; a tiny hole was made at its every corner.Such plastic container was then placed in direct contact with soil collected from the decomposition site within another larger circular plastic container for providing the natural environment for larval growth (Newton et al., 2005; Sing et al., 2012); filling up about 1/3 of its height. The set up described above was positioned in the middle of a shallow rectangular container that contained water for minimizing interruption that may be caused by fire ants on the growth of larvae. Using a slotted plastic basket with two bricks on top, the overall set up of the substrate was covered for curbing scavenger activities during decomposition. The wild-rearing fully grown third instar larvae of *C. megacephala* and *C. rufifacies* were sampled on day-4 and day-5 of decomposition, respectively. Considering that insects are poikilotherms, the ambient temperature data throughout the period of decomposition (day-1 to day-5) were recorded. The overall procedure described above was repeated for five sets of experiments for each of the type of beef substrate used.

Proximate Analysis of Nutrient Composition

The sample collected was killed using nearly boiling water (80˚C) (Adams and Hall, 2003), rinsed for three times with distilled water and dried on tissue papers. The sample was then dried at 100˚C for 24 hours in an oven, grounded into powdery form (Sing et al., 2014) and preserved at 4˚C prior to analysis. The sample was analysed for its proximate nutrient composition i.e. moisture, ash, crude protein, crude lipids, crude fibre and carbohydrate contents using the method suggested by theAssociation of Official AnalyticalCommunites(AOAC) (2000), optimized by the Institute of Bioproduct Development, IBD, UTM.

Statistical Analysis

The statistical analysis was performed using the IBM SPSS version 20 software. Prior to conducting the appropriate hypothesis testing, the normality of the data was ascertained by assessing the p-values indicated by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Considering that the data gathered in this present research proved to violate the assumption of normality, the non-parametric tests of Kruskal-Wallis with pairwise comparison using Mann-Whitney-U test was used for comparing the significant differences in medians (α=0.05) (Munro, 2005) among the different groups of larvae.

RESULTS

The larvae of *C. megacephala* and *C. rufifacies* were collected from decomposing beef muscle and liver substrates in a sunlit habitat within UTM, Johor Bahru campus. The fully-grown of third instar larvae of *C. megacephala* and *C. rufifacies* were consistently observed on day-4 and day-5 of decomposition, respectively. In general, the range of mean ambient temperature recorded at the decomposition site remained the same (26.0-28.0ºC) during the sampling months i.e. January and February 2015; with the daily ambient temperature ranged between 22-32ºC.

Proximate nutrient composition in the fully-grown third instar larvae of C. megacephala and C. rufifacies

The proximate analysis of the nutrient compositions in the wild-rearing fully-grown third instar larvae of *C. megacephala* and *C. rufifacies* is presented in Table 1. Irrespective of the types of beef substrates that those larvae fed on, significant differences in the proximate crude protein compositions between *C. megacephala* (beef muscle: 55.80 ± 6.21%; beef liver: 52.21 ± 2.90%) and *C. rufifacies* (beef muscle: 54.40 ± 2.60%; beef liver: 48.90 ± 8.41%) was not observed (Table 1). Furthermore, calculation on the proximate composition of carbohydrates revealed that *C. rufifacies* sampled from the decomposing liver substrateshad significantly higher median percentage (20.11 ± 9.44%) ($p<0.05$) than that of *C. megacephala* (10.50 ± 4.28%) (Table 1).

Significantly higher median percentage of crude lipids was observed in *C. megacephala* reared in beef muscle substrates (9.20 ± 0.45%) ($p<0.05$) when compared with that of *C. rufifacies* (4.68 ± 0.07%) (Table 1). In contrast, *C. rufifacies* that reared in beef liver substratescontained a significantly higher median percentage of crude lipids (5.50 ± 0.85%) ($p<0.05$) when compared with that of *C. megacephala* (2.74 ± 0.40%) (Table 1). Results revealed that *C. rufifacies* sampled from the decomposing liver substrates demonstrated a significantly higher median percentage of crude fibre (8.39 ± 0.64%) ($p<0.05$) when compared with that of *C. megacephala* (6.26 ± 0.45%) (Table 1). Furthermore, significantly higher medians of percentages of ash (7.96 ± 0.41%) and moisture (20.79 ± 1.08%) were observed in *C. megacephala* reared in beef liver substrates ($p<0.05$) when compared with those of *C. rufifacies* (i.e. 5.97 ± 0.26% and 10.38 ± 1.24%, respectively) (Table 1).

Table 1: Proximate analysis on the nutritional contents (dry matter) of the fully grown third instar larvae (wild-rearing) of *C. megacephala* and *C. rufifacies* sampled from beef muscle (n=5) and liver (n=5) substrates

	C. megacephala reared in beef muscle substrates	***C. megacephala*** reared in beef liver substrates	***C.rufifacies*** reared in beef muscle substrates	***C. rufifacies*** reared in beef liver substrates
Crude protein (%)	55.80 ± 6.21^{a} (46.95-62.40)	52.21 ± 2.90 (48.74-55.47)	54.40 ± 2.60^{b} (51.37-57.71)	48.90 ± 8.41 (43.46-57.14)
Crude fibre (%)	7.03 ± 2.01 (3.14-9.28)	6.26 ± 0.45 (5.78-7.07)	7.46 ± 0.75 (5.65-8.54)	$8.39 \pm 0.64^{b, d}$ (7.80-9.50)
Crude lipids (%)	$9.20 \pm 0.45^{a, c}$ (7.61-12.99)	2.74 ± 0.40 (2.16-3.51)	4.68 ± 0.07 (3.43-4.78)	$5.50 \pm 0.85^{b, d}$ (4.95-7.21)
Ash (%)	5.71 ± 0.17 (5.11-5.80)	$7.96 \pm 0.41^{a, d}$ (5.98-9.19)	$6.79 \pm 0.24^{b, c}$ (6.56-7.04)	5.97 ± 0.26 (5.72-6.78)
Moisture (%)	11.39 ± 0.77 (10.19-12.20)	$20.79 \pm 1.08^{a, d}$ (19.07-21.50)	$12.67 \pm 1.08^{b, c}$ (10.57-13.30)	10.38 ± 1.24 (9.65-11.31)
Carbohydrate (%)	12.40 ± 8.26 (6.01-19.30)	10.50 ± 4.28 (6.46-14.40)	14.61 ± 2.92 (11.74-17.58)	$20.11 \pm 9.44^{b, d}$ (12.25-25.48)

The lowercase superscripts (a, b, c and d) indicate the significant differences in the nutritional contents:

1. between the third instar larvae of *C. megacephala* reared in beef muscle when compared with that from beef liver (^{a})
2. between the third instar larvae of *C. rufifacies* reared in beef muscle when compared with that from beef liver (^{b})
3. between the third instar larvae of *C. megacephala* reared in beef muscle when compared with that of *C. rufifacies* from beef muscle (^{c})
4. between the third instar larvae of *C. megacephala* reared in beef liver when compared with that of *C. rufifacies* from beef liver (^{d})

Kruskal-Wallis with pairwise comparisons using Mann-Whitney U test was used for comparing the differences in the proximate nutrient compositions among groups (median ± interquartile range). Significance level of 0.05 was used for determining the significant differences among groups. The data in parentheses, ( ) indicate the range of the values.

Assessment on the suitability of beef substrates as the rearing media for C. megacephala and C. rufifacies

The proximate composition of crude protein in the third instar larvae of *C. megacephala* reared in both beef muscle and liver substrates ranged between 46.95-62.40% and 48.74-55.47%, respectively (Table 1). It was observed that *C. megacephala* reared in beef muscle substrates had a significantly higher median percentage of crude protein (55.80 ± 6.21%) ($p<0.05$) when compared with that reared in beef liver (52.21 ± 2.90%) (Table 1). Similarly, a significantly higher median percentage of crude protein was observed in *C. rufifacies* in beef muscle substrates (54.40 ± 2.60%) ($p<0.05$) when compared with that of liver (48.90 ± 8.41%) (Table 1). Furthermore, the median percentage of crude fibre in *C. rufifacies* in beef liver substrates (8.39 ± 0.64%) ($p<0.05$) was significantly higher than that of beef muscle (7.46 ± 0.75%) (Table 1).

It was evident that the median percentage of crude lipids in *C. megacephala* collected from beef muscle substrates was significantly higher (9.20 ± 0.45%) ($p<0.05$) when compared with that from the beef liver (2.74 ± 0.40%) (Table 1). In contrary, *C. rufifacies* that reared in beef liver substrates revealed higher median of crude lipids (5.50 ± 0.85%) ($p<0.05$) than that of in beef muscle (4.68 ± 0.07) (Table 1). Results on ash and moisture analyses revealed that *C. megacephala* from the liver substrates demonstrated significantly higher medians of percentages ($p<0.05$) when compared with that of in beef muscle (Table 1). Contrasting to the above finding, significantly higher medians of percentages ($p<0.05$) in ash (6.79 ± 0.24%) and moisture (12.67 ± 1.08%) were found in *C. rufifacies* reared in beef muscle substrates than that of in liver (ash: 5.97 ± 0.26%; moisture: 10.38 ± 1.24%) (Table 1). Results pertained to carbohydrates calculation indicated that *C. rufifacies* from beef liver substrates had significantly higher median (20.11 ± 9.44%) ($p<0.05$) when compared with that of in beef muscle (14.61 ± 2.92%) (Table 1).

DISCUSSION

Prevailing knowledge on the use of insects as animal feeds

Being an integral component in animal feed formulation, the demand for fishmeal as a source of protein has increased tremendously despite the decreasing availability of such natural resources (Barroso et al., 2014; Makkar et al., 2014; Sing et al., 2014). Studies have indicated that the fishmeal products contain about 59-73% of crude protein, 9.6-12.0% of crude lipids, about 0.9% of crude fibre, 16.6-19.2% of ash and about 7.3% of moisture (St-Hilaire et al., 2007; Kroeckel et al., 2012; Barroso et al., 2014; Sing et al., 2014; Makkar et al., 2014). In addition to its nutritional compositions, insects do not compete with humans for the same food resources since they can be reared on by-products and wastes generated by human and help in converting organic wastes into useful biomasses (Ramos-Elorduy, 1999; 2005). In this context, Diptera (true fly) has been the order of interest as possible candidates for complementing the sole dependency towards fishmeal products for agricultural sectors.

Despite requiring a long duration for completing its life cycle (Veldkamp et al., 2012), the use of *H. illucens* (larvae, prepupae and pupae) has been extensively reported in the literature (Barroso et al., 2014; Makkar et al., 2014). Previous studies (Ramoy-Elurduy et al., 1998; St-Hilaire et al., 2007; Odesanya et al., 2011; Kroeckel et al., 2012; Barroso et al., 2014; Makkar et al., 2014; Sing et al., 2014) have also reported about the proximate nutrient compositions in various species of dipterans (*viz. H. illucens*, *C. megacephala*, *Copestylumanna, Calliphoravicina, Drosophila melanogaster, Ephydrahians, Eristalis* sp., *Luciliasericata, Musca domestica* and *Protophormiaterraenovae*). Therefore, further studies for exploring the use of other insects such as *C. megacephala* and *C. rufifacies* that are prevailing in countries like Malaysia may prove useful.

It is pertinent to indicate here that while data on proximate nutrient compositions of *C. rufifacies* has never been reported in the literature; specific studies on *C. megacephala* within the same context remain scarce. While reporting about the proximate nutrient compositions in various dipterans, many studies did not provide clear indications on the rearing media used (Odesanya et al., 2011; Kroeckel et al., 2012; Barroso et al., 2014), the ambient temperature at which the insects grew (St-Hilaire et al., 2007; Odesanya et al., 2011; Kroeckel et al., 2012; Barroso et al., 2014; Ossey et al., 2014) and the instar stage of larvae analysed (Odesanya et al., 2011; Barroso et al., 2014; Ossey et al., 2014; Sing et al., 2014). Considering that those factors would affect the composition of nutrients in the larvae and since such information remains lacking in many papers, suitable comparisons and interpretation of the data may be difficult. In addition to higher composition of indigestible chitin among pupae (Murros et al., 2014), the third instar larvae of necrophagous insects have been reported as the most vigorous larval feeding stage with higher rates of absorption than that of elimination (Campobasso et al., 2004) as well as lower chitin composition (Kroeckel et al., 2012). Therefore, utilization of the fully-grown third instar larvae for animal feed formulation may prove feasible. In this context, this study that investigated the proximate nutrient compositions of the wild-rearing third instar larvae of *C. megacephala* and *C. rufifacies* in beef muscle and liver substrates decomposing in a sunlit habitat, merits consideration.

Comparison between the proximate nutrient composition in the fully-grown third instar larvae of C. megacephala and C. rufifacies

Studies have indicated that insects such as *C. megacephala* and *C. rufifacies* are unable to maintain their body temperatures (poikilotherms) and hence, depend on the ambient external heat for sustaining their physiological processes (Gennard, 2007; Rivers and Dahlem, 2014). In general, the growth rate of such insects has been reported as positively correlated with the ambient temperature (Rivers and Dahlem, 2014). Unfortunately, such an important information as the ambient temperature remains unreported in many studies on proximate nutrient compositions in insects (St-Hilaire et al., 2007; Odesanya et al., 2011; Kroeckel et al., 2012; Barroso et al., 2014; Ossey et al., 2014), rendering difficulties in making the appropriate comparisons.

Due to the similarity in the ambient temperature recorded throughout the sampling periods and since the fully-grown third instar larvae of *C. megacephala* and *C. rufifacies* were invariably observed on day-4 and day-5 respectively, it can be construed that all the larvae analysed were uniform in growth. The observations of the third instar larvae of *C. megacephala* and *C. rufifacies* in those days of decomposition appear to be consistent with that reported by previous researchers (Omar et al., 1994; Mahat et al., 2009; 2014). Such a consistency in growth observed in all the larvae analysed would enable suitable comparisons to be made in the proximate nutrient compositions among the different groups.

Interestingly, the ranges of median percentage for crude protein composition in *C. megacephala* and *C. rufifacies* found here were observably higher than that of the larvae of *H. illucens* (36.2-42.1%) (Newton et al., 1977; Ramos-Elorduy, 1998; Arango Gutierrez et al., 2004; Barroso et al., 2014), signifying the high potential of these two species as protein sources. The fact that statistically significant differences were not observed between *C. megacephala* and *C. rufifacies* obtained from both the beef muscle and liver substrates, it can be suggested that both species may be equally useful as protein sources. The proximate crude protein compositions in *C. megacephala* and *C. rufifacies* reported here were lower than that of the commercially available fishmeal products (58.7-73.0%) reported in the literature (St-Hilaire et al., 2007; Kroeckel et al., 2012; Barroso et al., 2014; Makkar et al., 2014; Sing et al., 2014). However, the crude protein compositions found in these two necrophagous species appear to be adequate for broiler chickens (18-23%), layer chickens (12.5-18.8%), turkeys (14-28%) and ducks (15-22%) (United States Department of Agriculture, USDA, 2003). The fact that catfish also requires about 25-50% of dietary protein (Robinson et al., 2001), the amount of protein found in the third instar larvae of *C. megacephala* and *C. rufifacies* prove to be sufficient too.

It was evident that the median of proximate carbohydrate compositions in the third instar larvae of *C. rufifacies* reared in beef liver substrates were significantly higher than that of *C. megacephala* ($p<0.05$). Despite being statistically insignificant ($p>0.05$), similar pattern in the proximate composition of carbohydrate was observed in *C. rufifacies* in beef muscle substrates than that of *C. megacephala*. Higher proximate composition of carbohydrate observed in the third instar larvae of *C. rufifacies* may be due to its ability to store more carbohydrate than that of *C. megacephala* and this aspect should form an interesting study. Notwithstanding, the medians of carbohydrate compositions in *C. megacephala* and *C. rufifacies*reported in this study were considerably higher than that reported in fishmeal products (0.8-9.5%) (Awoniyi et al., 2003; Kroeckel et al., 2012; Barroso et al., 2014) as well as in the larvae of *C. megacephala* (0.75-4.00%) reported by previous researchers (Sing et al., 2014; Barroso et al., 2014). Being the immediate source for energy, higher contents of carbohydrate observed in these two species when compared with fishmeal productsdemonstrated their practical values in complementing the nutrients required for animal feeds.

Significantly higher median of crude lipids in *C. megacephala* collected from beef muscle substrates was observed when compared with that of *C. rufifacies*. In contrast, significantly higher crude lipids content was observed in the *C. rufifacies* in beef liver substrates than that of *C. megacephala*. Such contrasting findings reported here concur with the indication made by Barroso et al. (2014) that 'the lipids content varies enormously' and 'it is difficult to obtain a clear conclusion regarding relation between lipids content and taxon, stage or feeding'. Therefore, further studies for providing suitable explanations to such variations are required. It has been indicated that lipids play important roles in animal's metabolism *viz.* supplying essential fatty acids (Omega-3 and Omega-6), a vehicle for absorbing fat-soluble vitamins as well as precursors for steroid hormones and other compounds (Robinson et al., 2001). Although excessive fat in animals may reduce the processing yield, quality and storage of processed products (Robinson et al., 2001); such high contents of lipids observed in these two species may be useful for other scientific purposes. In this context, Zheng et al. (2013) indicated about the possible use of oil from insect's larvae for producing biodiesel. The fact that the lipids may also contain essential fatty acids such as Omega-3 and Omega-6 (Robinson et al., 2001), its extraction for animal feed formulations may prove useful.

Results of this present study revealed that the medians of crude fibre compositions in all the specimens of *C. megacephala* and *C. rufifacies* ranged between 6.26-7.03% and 7.46-8.39%, respectively. In addition, the median of crude fibre composition in of *C. rufifacies* reared in beef liver substrates was significantly higher than that of *C. megacephala*. Such higher compositions of crude fibre observed in *C. rufifacies* than that of *C. megacephala* can be attributed to predatory behaviour of the third instar larvae of *C. rufifacies* (Bharti and Singh, 2003; Ahmad and Ahmad, 2009).The results appear to be comparable with the compositions of crude fibre reported among the different species of Diptera (5.89-15.70%)(Newton et al., 2005; Odesanya et al., 2011; Pretorius, 2011; Ossey et al., 2014) although higher than that of in fishmeal products (0.9%)(Odesanya et al., 2011). While Robinson et al. (2001) indicated that animals with a single gastric stomach such as catfish and chickens would not be able to derive any direct nutritional benefits from the consumption of dietary fibre, ruminants such as cows may still benefit from the high content of fibre in the two necrophagous species. In concurrence with the statement made by Barroso et al. (2014) that insects had less ash than that of fishmeal, in this study too, lesser contents of ash (5.71-7.96%) in all the *C. megacephala* and *C. rufifacies* analysed were observed.

Suitability of beef muscle and liver tissues as rearing substrates for C. megacephala and C. rufifacies third instar larvae.

Review of the literature reveals that the proximate compositions of beef muscle and liver tissue vary among the different strains and locations of breeding detailed below. It has been indicated that the beef muscles of cows in Malaysia contain about 28.2% of protein, 9.41% of lipids and undetectable amount of carbohydrate (Department of Veterinary Services and Animal Industry Sabah, 2015, personal communication). While reviewing the composition of selected nutrients in beefs and lambs in Australia and New Zealand, Williams (2007) reported about differences in nutrients in beef muscle (about 73%, 23%, and 3% of moisture, protein and fats, respectively) and liver tissues (about 20% and 9% of protein and fat, respectively). In a study conducted in Nigeria, Adeniyi et al. (2011) reported that the beef muscle tissue of the *Longissimus dorsi*contained about 93% of crude protein, 5% of lipids contents, 2% of carbohydrate, 0.4% of ash and undetectable amount of crude fibre. Moreover, the beef muscle of Norwegian Red cattle has been reported to contain about 3% of fat, 19% of total amino acid, 1% of ash and 74% of water (Jensen et al., 2014). While studying liver samples of *Qinchuan* cattle and crossbred cattle, Li et al. (2014) indicated that the two strains varied in proximate compositions of nutrients. The *Qinchuan* liver samples contained about 74% of moisture, 17% of crude protein, 3% of fat, 4% of carbohydrate and 1% of ash (Li et al., 2014). On the other hand, the crossbred liver samples contained 72% of moisture, 19% of protein, 5% of fat, 3% of carbohydrate and 1% of ash (Li et al., 2014).

Results of this study revealed that *C. megacephala* reared in beef muscle substrates contained significantly higher crude protein ($p<0.05$) when compared with that of in beef liver substrates. Similarly, the crude protein in *C. rufifacies* in beef muscle substrates was significantly higher ($p<0.05$) than that of in beef liver substrates. Significantly higher crude lipids composition was observed in *C. megacephala* in beef muscle substrates ($p<0.05$) when compared with that of in beef liver substrates. Moreover, *C. rufifacies* in beef muscle substrates demonstrated significantly higher composition of crude lipids ($p<0.05$) than that of in beef liver substrates. Such high compositions of crude protein and crude lipids found in the larvae that fed on beef muscle substrates can be attributed to high protein (23-93%) and lipids (3-9%) compositions within the beef muscles reported by the previous researchers (Williams, 2007; Adeniyi et al., 2011; Department of Veterinary Services and Animal Industry Sabah, 2015, personal communication).

In contrast to the patterns observed for crude protein and crude lipids, significantly higher carbohydrate composition was observed in *C. rufifacies* collected from beef liver substrates ($p<0.05$) when compared with the same species in beef muscle. The same pattern observed for carbohydrate was also prevailed for crude fibre; significantly higher crude fibre composition in *C. rufifacies* in beef liver substrates ($p<0.05$) than that of in beef muscle. It has been reported that the composition of carbohydrate in liver samples of two different breeds of cattle ranged between 3-4% (Li et al., 2014). Since beef muscles contain limited amounts of carbohydrate (0-2%) and fibre (0%), while predatory behaviour towards *C. megacephala* has been widely reported, higher compositions of these two elements observed in the larvae of *C. rufifacies* reared on beef liver substrates proves not unexpected.

CONCLUSION

Results of this study revealed that the third instar larvae of *C. megacephala* and *C. rufifacies* reared on both types of beef substrates contained substantial amounts of crude protein, crude lipids, carbohydrate and crude fibre, which were comparable to and/or better than that of *H. illucens*. Although crude protein composition in these two species was found to be lower than that of the commercially available fishmeal products, the amount of protein observed was evidently sufficient for sustaining the growth for a number of livestock commodities such as broiler chickens, layer chickens, turkeys and ducks. Hence, such a situation advocates on the practical use of these two prevalently found necrophagous species in Malaysia for animal feeds.It was found that the use of beef muscle as rearing substrates would facilitate the attainment of maximum composition of crude protein and crude lipids in the third instar larvae of these two species. In contrary, the use of beef liver substrates has resulted in optimum composition of carbohydrate and crude fibre. Therefore, this study successfully demonstrated that both of the beef muscle and liver substrates complemented each other in attaining the optimum amounts of nutrients in the third instar larvae of *C. megacephala* and *C. rufifacies*.

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