The Effect of Dietary Fat Levels on the Size and Development of *Chrysomya megacephala* (Diptera: Calliphoridae)

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**ABSTRACT.** Variation in the type of tissue that larvae feed on can produce marked differences in developmental rate and body size, which can compromise predictions of minimum postmortem interval. A series of experiments were conducted to investigate the effect of fat content in the diet on larval growth in *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae), an important forensic blowfly species in China. Bioniomical parameters such as body size, development time, mortality, and sex ratio were observed. The results indicated that fat content in the diet has a dramatic effect on the body size and larval development. More dietary fat content was beneficial for development of larvae in first and early second instar. But it was adverse in the later third instar. Significantly, a high-fat diet resulted in increased development rates and the production of undersized larvae and adults. Overall mortality of larvae and pupa was higher when more fat was added to the diet, but sex ratio of adults was not negatively affected. This study highlights that the fat content in the diet should be considered in the entomological research and forensic application when estimating minimum postmortem interval on the basis of larval body size and developmental stage.

**Key Words:** entomology, *Chrysomya megacephala*, postmortem interval, development

It is common in practical applications of forensic entomology to consult data on the development of flies when estimating the minimum postmortem interval (PMI$_\text{min}$). Forensic entomologists estimate the minimum time between death and discovery of a corpse mainly in terms of the parameters of body size and developmental stage of blowflies which are found in or on a corpse. Current standard development rate curves of blowflies are produced by extrapolation from analysis of development rate reared on a known single or semisynthetic substrate under controlled environmental conditions. Many varied organs or bodies of animals, especially mammals, have been used in larval growth and succession studies (Byrd and Butler 1996; Grassberger and Reiter 2001, 2002; Ames and Turner 2003; Grassberger et al. 2003; Kaneshrajah and Turner 2004; Clark et al. 2006; Day and Wallman 2006a; Ireland and Turner 2006). In some cases, artificial diets were also recommended (Mandeville 1988, Daniels et al. 1991, Rabelo et al. 2011).

Previous experiments on the development of blowfly larvae showed that the components and nutrition of the diet play a key role in larval development. Specifically, the composition of the tissue that the larvae fed on had significant effects on development. These differences were dependent on the fat/protein ratios and carbohydrate levels of different tissue substrates (Kaneshrajah and Turner 2004, Clark et al. 2006, Day and Wallman 2006a, Ireland and Turner 2006, Arong et al. 2011). An error of almost 2 d could be expected in a PMI$_\text{min}$, estimate using *Calliphora vicina* larvae reared on liver compared with other organs, such as brain, heart, kidney, and lung (Kaneshrajah and Turner 2004). Similar results were obtained in other blowflies, such as *Lucilia sericata*, *Calliphora augur*, and *Lucilia cuprina* (Clark et al. 2006, Day and Wallman 2006a). As fatty substances are generally used and stored for energy, larvae consuming more fat will be able to direct more energy into growth (Day and Wallman 2006b). For this reason, the fat content in diet may affect the growth and development of blowfly and these factors must be accounted for in the entomological research or in estimates of PMI$_\text{min}$.

Fat tissue is a very important part of the body and an indispensable component in the diet of the necrophagous species, especially the larvae of blowflies. Studies on the effect of nutrient composition and fat content in the diet on larval growth of the blowfly are sparse in the forensic entomology literature. The aim of this study, therefore, was to observe whether the fat content in the diet effects body size and developmental stage of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae). The blowfly *C. megacephala* was selected for these experiments because it is the most dominant species that infests carcasses in local crime scenes in most areas of China (Liu et al. 2009). Moreover, it was also one of the first saprophagous organisms to arrive and lay eggs on a body after death.

**Materials and Methods**

**Diet Preparation**

Fresh pure lean pork and subcutaneous fat obtained from an abattoir on the day of slaughter from different pigs. The diet for the experiment was a mixture of pure lean pork and subcutaneous fat, including five groups according to the different proportions of fat content. The proportion of fat in the mixture was 0% (pure lean pork, control treatment), 10, 30, 50, and 80% by weight in the mixture diet and labeled G0, G1, G3, G5, and G8, respectively. The mixture was prepared by mincing by a liquidizer. Each diet was 200 g and stored at $-20^\circ$C for use during the five replicates of the experiment. The diets were thawed and equilibrated to room temperature before the egg mass was migrated.

**Blowfly Maintenance**

The strain of *C. megacephala* used in this experiment was established cultures originating from individuals trapped in Chongqing (29° 59′ N; 106° 54′ E), China. These flies were inbred for four generations
to obtain purebred and an adequate number of flies for the subsequent experiments. About 200 flies were maintained in rearing cages (100 by 100 by 100 cm) and the ratio of males to females was held at ~1:1. Growing cultures were supplied with food consisting of water, sugar, and milk powder. Fresh pork blood was added in the food to promote the development of the ovaries of female adults beginning 3 d after eclosion. The rearing cages were maintained in the laboratory at an approximate temperature of 26 ± 3°C.

When eggs were required, the cages were presented with sliced fresh pork liver (3 by 10 by 10 cm) for 1 h to oviposit. Egg masses were collected from the pork liver and 200 eggs were transferred to each diet as outlined above. The diet was sufficient for completing the larval stage according to our pilot experiments and previously published data conducted in three forensic blowfly species (Daniels et al. 1991, Saunders and Bee 1995, Ireland and Turner 2006). Each diet was placed in a 500-ml beaker (diameter, 8.5 cm; height, 12 cm) and each beaker was placed inside a rearing cage (30 by 30 by 40 cm). The bottom of each rearing container was covered with a 2-cm-deep layer of sawdust to provide the larvae a favorable place to pupate. Adequate mesh ventilation was provided through the transparent lid. All the rearing containers were kept in a versatile environmental test chamber (MLR-351 H, Sanyo, Osaka, Japan) maintained at a constant temperature of 25 ± 0.3°C, 75 ± 5% relative humidity (RH), and a photoperiod of 14:10 (L:D) h throughout the experiment.

**Measurement, Data Handling, and Statistical Analysis**

Observations were performed at 12-h intervals and consisted of destructive sampling. The first time was performed 16 h after egg hatching. A sample of 10 randomly chosen larvae were collected at each time point until larvae began to reach the postfeeding stage. To prevent disturbance of the growing larvae, the time spent searching for larvae in each group was limited to 15 min, as described in Day and Wallman (2006a) and Arong et al. (2011). The samples of larvae were washed quickly with distilled water to remove adhering diet and killed by immersion in 90°C water for 30 s dried with paper towels before the measurement. In total, 10 dark brown pupae of each group and left wings of 10 adult blowflies were also collected for subsequent measurements. The samples of pupae were collected in the first observation when the pupae turned to be dark brown.

Body weight of the larvae and pupae was collected individually by an electronic analytical balance with an accuracy of ±0.1 mg (AUW 220D, Shimadzu Corporation, Kyoto, Japan). The length of larvae between the most distal part of the head and the last abdominal segment and the length of pupae were measured using a digital micrometer with an accuracy of ±0.001 mm (Mitutoyo, Kawasaki, Japan). The size of each adult was determined, as in other studies (Clark et al. 2006, Ireland and Turner 2006), by measuring the length of posterior cross vein (dm-cu) on the left wing by means of a microscope equipped with a digital camera (Leica CH-9435, LAS Version 3.8.0, Leica Microsystems, Switzerland) at a magnification of 20×.

The time of larvae wandering and adult emergence was recorded and the number was also counted at 12-h intervals for subsequent statistical analysis of time of developmental stage. The duration of each developmental stage was expressed by mean ± standard deviation (mean ± SD) in hours. All of the dead larvae, pupae, and puparium in the sawdust were collected and recorded to calculate the mortality rate of larvae and pupae. Adult blowfly sex was measured according to the presence or absence of a gap between their eyes. Adult sex ratios of number of female: (female + male) were recorded.

Each treatment of different diet groups was repeated five times. There was no significant difference in larval length and weight, pupal length and weight, cross-vein length, and developmental duration between any of the five replicated tests (P > 0.05 in all comparisons), and hence the data were pooled for subsequent statistical analysis. The data were analyzed with the statistical software SPSS 18.0 (SPSS Inc., Chicago, IL). One-way analysis of variance was used to consider differences in size of larvae, pupae, and adult blowfly and the duration between different groups. Numerical differences are reported in the text as mean values with 95% confidence intervals.

**Results**

**Larval Length.** Our results showed that larval body length increased markedly from 16 to 40 h after hatching for G1, G3, G5, and G8 diet groups compared with the G0 control group (P < 0.001). Nevertheless, the pattern was reversed when time went on and the body length varied inversely at 64 h. This phenomenon was sustained throughout pupation until eclosion. Greater fat content in the diet resulted in smaller-sized individuals in length. In addition, the mean maximal length was reached in 88 h for the G0, G1, G3, and G5 groups, whereas the mean maximal length for G8 was not reached until 100 h. Larvae began to reduce their body length in preparation for pupation after mean maximal length time. A statistically significant difference was observed in the mean maximal length between the five groups (P < 0.001). The length growth curves of larval samples collected from each different treatment are shown in Figure 1 and Table 1.

**Larval Weight**

Increased dietary fat also had a significant effect on larval body weight. The body weight of larvae fed a high-fat-content diet was heavier in early larval stage from 16 to 28 h than that of control group (P < 0.001). However, it is worth noting that the pattern was reversed after 40 h and more fat content in the diet produced lighter individuals. The phenomenon was sustained until postfeeding stage and pupae stage. The mean maximum weight for G0, G1, G3, and G5 was reached in 88 h and that for G8 was not reached until in 100 h, after which a slight decrease occurred as larvae entered the postfeeding stage. A significant difference between different treatments can be found in the mean maximum body weight (P < 0.001) related to the fat content in the diet. The weight growth curves of larval samples collected from the different diets are shown in Figure 2 and Table 1.

**Pupal and Adult Body Size.** The body length and weight of pupae related to the level of fat content in the diet. This effect is significant between the five dietary groups (P < 0.001; Table 1). The smallest mean length and weight of pupae were found in G8 and the largest mean length and weight of pupae were found in G0. The length of posterior cross vein (dm-cu) on the left wing of adults between different groups was also affected. There was a significant difference in the length of the posterior cross vein between the five groups (P < 0.001).

**Fig. 1.** Larval growth (length) from 16 to 112 h after egg hatching on different levels of fat content in diet. Length growth curves are different between the five treatments. More dietary fat content resulted in the longer larval length from 16 to 40 h and shorter larval length from 52 to 112 h.
Different between the five treatments. More dietary fat content different levels of fat content in diet. Weight growth curves are significantly different within the same stages of development.

Fig. 2. Larval growth (weight) from 16 to 112 h after egg hatching on different levels of fat content in diet. Weight growth curves are different between the five treatments. More dietary fat content resulted in the heavier larval weight from 16 to 40 h and lighter larval weight from 52 to 112 h.

Development. The duration of the third instars and total development time of high-fat-content diet treatments (G5 and G8) were shorter than the other three treatments (P < 0.01; Table 2), whereas there was no significant difference in the duration of pupae between the five groups (P > 0.05). The effect of larval developmental time also differed individually in the high-fat-content group. The time to enter the postfeeding stage more widely varied among individuals with the G8 treatment. Larger standard deviation was found in time of third instar and total development time.

Mortality and Sex Ratio. Fat content in the diet influenced larval mortality significantly (P < 0.001; Table 3). The highest larval mortality occurred on the high-fat-content diet of G8, whereas the lowest larval mortality occurred on the G0 diet control group. There was no significant difference in larval mortality between G0, G1, and G3 (P > 0.05). The pupal mortality of G5 and G8 was higher compared with other three groups (P < 0.001), and the lowest emergence rate was observed in G8. There were no significant differences in emergence rate of pupae between the three groups of G0, G1, and G3 (P > 0.05). The mean adult sex ratio ranged from 47 to 52%. There were no significant differences observed in the sex ratio between the five groups reared on different diets (P > 0.05; Table 3).

Discussion

The development of necrophagous insects colonizing a corpse can be influenced by many environmental factors. Ordinarily, the ambient temperature of the scene where the body is found would be considered the primary factor (Byrd and Butler 1996, Anderson 2000, Grassberger and Reiter 2002, Ireland and Turner 2006, Niederegger et al. 2010). Other important factors that have been reported are larval population density (Goodbrod and Goff 1990, Saunders and Bee 1995, Saunders et al. 1999, Ireland and Turner 2006), photoperiod (Nabity et al. 2007), rain (Mahat et al. 2009), and the presence of drugs in the body tissue (Introna et al. 2001, Mahat et al. 2009). As one of the most important biochemical components and an essential nutrient in the diet, lipids can provide crucial energy and assist the organism in coping with environmental variation and the competing demands of growth, survival, and reproduction (Ujvari et al. 2009). In the complex life cycle of holometabolous insects, the last few days of larval development are characterized by the accumulation of nutrient reserves in the larval body for use during the subsequent developmental stages (Ujvari et al. 2009). The fat cells carried over from the larval stage, through pupation to adulthood, have been shown to have a nutritional role in the early, nonfeeding stage of adulthood (Aguila et al. 2007). Apart from being an energy source, lipids have many additional functions such as preventing desiccation and in chemical communication (Hulbert et al. 2005).

Five replicated experimental studies in this study have shown that more dietary fat was very beneficial for development of larvae that were in the first and early second instar. A previous study also showed that moderate amounts of fat are required in promoting growth among blowfly larvae (Vanderzant 1974). However, fat was an adverse factor in the later third instar. This is supported by the previous study that low levels of dietary fat enhanced blowfly lifespan and high-fat diets had a detrimental impact on blowfly physiology (Ujvari et al. 2009). High oleic acid diets have also been found to kill fourth-instar larvae mosquito (Rahuman et al. 2008). Taken together, these data indicate that high-fat content in the diet may be an important obstacle for the larval development at the later stages of third instar. Moreover, it is likely to lead to increased development rates and more smaller-sized individuals.

The different levels of fat content in the diet not only affected the larval body size but also had a profound effect on the developmental stage of blowfly. This may indicate that larvae would migrate or pupate prematurely rather than slow development if there is no suitable diet to feed on. Therefore, an increased developmental rate can also mislead the forensic practitioner during casework and result in an overestimate of the PMImin (Ireland and Turner 2006). The level of fat content in the diet has relation with the larval behavioral ecology of C. megacephala. A small proportion of fat in the diet would not affect the larval development rate. A great deal of fat in the diet would provide insufficient nutrition or would be adverse for third-instar larval development or even result in hypoplasia in individuals, despite the slight benefit seen in the early larval stages.

The results of this study suggest that fat content in the diet has a profound effect on larval body size of C. megacephala. As discussed above, the body size of the larvae reared on a diet containing more fat was dramatically smaller than those reared on a diet of lean pork in the later larval stage of third instar. In summary, there was a significant difference in the body length and weight in those reared on the diet

| Table 1. Body size of *C. megacephala* in different development stage reared on diets of different fat content |
|---|---|---|---|---|---|
| Diet | Length (mm) | Wt (mg) |
| | First-instar larva in 16 h | Second-instar larva in 28 h | Third-instar larva in 88 h | Pupae | Crossoverin |
| | First-instar larva in 16 h | Second-instar larva in 28 h | Third-instar larva in 88 h | Pupae |
| G0 | 4.2 ± 0.2a | 6.8 ± 0.3a | 17.4 ± 1.3a | 10.2 ± 0.5a | 1.80 ± 0.04a | 1.0 ± 0.1a | 4.6 ± 0.3a | 94.4 ± 13.1a | 69.5 ± 10.5a |
| G1 | 4.3 ± 0.2b | 6.9 ± 0.2a | 16.2 ± 1.2b | 9.4 ± 0.3b | 1.66 ± 0.04b | 1.2 ± 0.1b | 5.8 ± 0.3b | 78.8 ± 12.1b | 56.3 ± 6.4b |
| G3 | 4.4 ± 0.2c | 7.2 ± 0.4ab | 14.7 ± 1.1c | 8.7 ± 0.5b | 1.52 ± 0.05c | 1.2 ± 0.1b | 6.5 ± 0.2c | 63.4 ± 8.7c | 39.6 ± 6.4c |
| G5 | 4.4 ± 0.3cd | 7.5 ± 0.4bc | 13.2 ± 0.9d | 7.9 ± 0.7c | 1.37 ± 0.12d | 1.3 ± 0.2c | 7.9 ± 0.5d | 45.2 ± 6.3d | 34.2 ± 7.3cd |
| G8 | 4.5 ± 0.4d | 7.8 ± 0.5c | 11.2 ± 0.9e | 7.4 ± 0.6c | 1.21 ± 0.13e | 1.4 ± 0.1d | 8.7 ± 0.4e | 31.2 ± 4.2e | 29.1 ± 7.7d |

G0, G1, G3, G5, and G8 mean containing fat tissues 0, 10, 30, 50, and 80% in weight, respectively. Mean values followed by the same letter are not significantly different within the same stages of development.
containing a different amount of fat tissues in weight. More fat content in the diet produced undersized postfeeding larval individuals. At later stages, the smaller larvae gave rise to smaller pupae and subsequently smaller adult blowflies. As the body size of fly larvae collected from a corpse is one of the most frequently measured parameters for success-ful estimating PMImin, the results may have an important implication in that the smaller-sized larvae may be mistaken for younger individuals by forensic practitioners and result in an underestimated time of death.

However, the nutritional intake of larvae is likely to vary subject to the part of a corpse on which they are feeding as a natural cadaver will have areas of fat and meat that they can choose between (Day and Wallman 2006a). Larvae can choose their rearing substrate from a nutritional viewpoint (d’Almeida and Salviano 1996), and dietary self-selection by insects is a widely observed phenomenon (Waldbauer and Friedman 1991). To observe the effect of fat content in the diet on larval growth accurately, the mixture of diet in this study was prepared by mincing in a blender so that the larvae could not make choices between fat and meat during the period of feeding. Therefore, although our results show that dietary fat is an important factor, the effects on rates of development would likely be different if they were fed on natural cadavers that allowed self-selection. An inaccurate estimation would be made in terms of the data derived from a single type of animal tissue rather than that on which larvae at a death scene have been feeding as the body size and developmental rate of blowflies may be different due to feeding on different tissues under natural conditions (Kaneshrajah and Turner 2004, Clark et al. 2006).

Our findings have potential implications for forensic entomologists by showing that the fat content should be taken into account more explicitly in laboratory experiments, because raw meat or organs from domestic animals are commonly used in development studies of necrophagous species. The lack of standardized procedures among laboratories often compromises extrapolation of the findings to different forensic scenarios (Rabelo et al. 2011).

Fat tissue is easier for larvae to break down and consume (Day and Wallman 2006a). This may be an important cause of the acceleration of larval development in the earlier stages. However, the liquefied fat of the degradation products in feeding may be another important factor influencing the larval activity and ingestion in the later larval stage. Therefore, it seems that the main factor contributing to the differences in body size and developmental rate of the larvae observed in this study are nutrients in the diet, but tissue structure may also play a role.

Length and width of puparal cases are helpful to discriminate between sarcophagids and calliphorids according with their own diagnostic features (posterior spiracles, in particular) though it is not enough for species identification (Smith 1986, Mazzanti et al. 2010). However, there was a significant difference in body length and weight of pupae and the length of posterior cross veins on the left wing that represented the body size of adults. It was evident that the body size can also be, to some extent, dependent on food availability.

There was a significant difference in larval mortality and pupal mor-tality between the five groups reared on different diets. The larval mor-tality and pupal mortality of C. megacephala increased directly with the increased fat content in the diet. High larval and pupal mortality were seen in G5 and G8. However, the sex ratio of adults of C. megaceph-ala ranged from 47 to 52% and was not affected by fat content in the diet. The results were similar to other calliphorid species, such as C. megacephala and Chrysomya putoria reared on different semisynthetic diets in previous studies (Mendonca et al. 2009, Rabelo et al. 2011).

Conclusions. In conclusion, our results show profound effects of variations in dietary fat content on larvae development rate and body size of C. megacephala. A high-fat diet was beneficial for development of larve in first and early second instar but it had an adverse effect in the later third instar. Our results also revealed that larvae feeding on a diet containing more fat would migrate prematurely and reached the wan-dering phase significantly earlier than those fed on a diet with less fat. This study highlights that the fat content in the diet should be considered. In addition, the position and composition of larvae feeding should be taken into account more explicitly at a death scene when estimating PMImin on the basis of larval body size and developmental stage, espe-cially the case of an obese or dismembered carcass. The results suggest that further experiments are required in order to understand how dietary fat is metabolized and utilized by blowfly larvae.

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**Table 2. Time of developmental stage (h) of C. megacephala reared on diets of different fat content**

| Diet  | Time of first instar (n) | Time of second instar (n) | Time of third instar (n) | Time of pupae (n) | Total development time (n) |
|-------|--------------------------|--------------------------|-------------------------|------------------|---------------------------|
| G0    | 16.0 ± 0a (50)           | 24.0 ± 0a (50)           | 78.5 ± 18.7a (523)      | 124.3 ± 12.5a (451) | 242.8 ± 27.4a (451)       |
| G1    | 16.0 ± 0a (50)           | 24.0 ± 0a (50)           | 81.7 ± 18.6a (544)      | 123.8 ± 14.6a (442) | 245.5 ± 29.8a (442)       |
| G3    | 16.0 ± 0a (50)           | 24.0 ± 0a (50)           | 79.9 ± 19.8a (535)      | 122.7 ± 13.2a (416) | 242.6 ± 26.5a (416)       |
| G5    | 16.0 ± 0a (50)           | 24.7 ± 2.9a (53)         | 64.0 ± 21.4b (369)      | 125.4 ± 17.1a (251) | 230.1 ± 32.2b (251)       |
| G8    | 16.0 ± 0a (50)           | 25.0 ± 3.3a (54)         | 57.7 ± 24.5c (230)      | 126.5 ± 18.4a (120) | 225.2 ± 42.8b (120)       |

G0, G1, G3, G5, and G8 mean containing fat tissues 0, 10, 30, 50, and 80% in weight, respectively. Mean values followed by the same letter are not significantly different within the same stages of development. Total development time includes the duration time of larva and the duration time of pupa.

**Table 3. Bionomic parameters of C. megacephala reared on diets of different fat content**

| Diet  | Mortality larvae (%) | Mortality pupae (%) | Sex ratio (%) |
|-------|----------------------|---------------------|---------------|
| G0    | 5.5 ± 3.1a (500)     | 4.6 ± 1.5a (473)    | 48.2 ± 4.3a (451) |
| G1    | 4.7 ± 2.2a (500)     | 6.8 ± 2.4a (477)    | 51.4 ± 4.1a (442) |
| G3    | 9.7 ± 3.5a (500)     | 7.9 ± 3.1a (452)    | 52.8 ± 3.2a (416)  |
| G5    | 31.4 ± 4.7a (500)    | 26.8 ± 5.5b (343)   | 47.7 ± 3.2a (251)  |
| G8    | 58.3 ± 8.2c (500)    | 42.5 ± 7.2c (208)   | 50.1 ± 4.9a (120)  |

G0, G1, G3, G5, and G8 mean containing fat tissues 0, 10, 30, 50, and 80% in weight, respectively. Mean values followed by the same letter are not significantly different within the same stages of development. n-value is given in parenthesis.
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