4-17-2013

A Comparison of Common Diets for the Laboratory Culture of the Green Bottle Fly, Lucilia sericata

A comparison of common diets assessed by the survivorship and fecundity of *Lucilia sericata* (Diptera: Calliphoridae)

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Introduction

Adult and larval species of *Lucilia sericata* are known for their importance in forensic entomology as a primary colonizer of decomposing remains and are important in medicine for their roles in human wound debridement and myasis. Hobson developed a complex nutritive food source for colony maintenance and was further streamlined in 2001 by Tachibana and Numata. Some laboratories still choose to follow this recipe to make a complex food source for adult flies, but others are choosing to feed colonies only essential dietary components. This poster compares two sugar sources within three diets to discover which provides for the best feeding, breeding, and rearing outcome for both forensic entomology and maggot debridement therapy (MDT) colonies.

The most common contribution of forensic entomology is the establishment of a PMI, or post-mortem interval, by staging the insect larvae found at the scene (Anderson 200, Zurwaski et al. 2009, Arnaldos et al. 2005, Huntington et al., 2007). It is easy to estimate the amount of time a victim has been exposed to the elements, once the age of the larvae is established. The time it takes for the movement of blow fly larvae through the three instar stages to pupae is well established, under a specific temperature and environmental conditions (Browne 1993, Campobasso 2001).

Criminal cases hinge on the work done at the bench and seeing the importance such data in law proceedings, the *Daubert v. Merrell Dow Pharmaceuticals* decision mandated scientific evidence to be testable, have a known error rate, to be peer-reviewed and accepted practice within the scientific community. A renewed importance is now placed on the methods and protocols used in the lab to establish colonies from field-gathered species. It is necessary for consistency to exist between labs performing any type of molecular or microbial forensic analysis, if specimens are being analyzed in more than one lab. Pursuing to increase the ease of laboratory processes of raising flies, we tested three of the most commonly used diets to see how each affected the flies' lifespan and fecundity.

Materials and Methods

Once the larvae pupated, 1200 pupa were separately place in 1 oz cups capped with breathable lids. All were placed in a Powers Scientific Incubator that was kept at a 40 +/- 2% humidity, 28˚C, with a 12hr light-dark cycle. The adult flies were sexed and placed into Bug Dorms with 12-20 females and 12-20 males, for a total of 24-40 flies in each Bug Dorm. Each Bug Dorm of flies emerged within 12 hours of each other that came from the same batch of larvae. A total of three cages of flies were assigned to one of the nine respective diets, as detailed in Fig. 1. Flies were subjected to their respective diets within 2 hours of being separated and caged. All of the Bug Dorms were kept in a portable fly enclosure that maintained temperature at 23 +/- 3˚C, a humidity of 35+/- 3%, with a 12 hour light cycle, which was monitored by a HOBO measurement device.

Flies were given one of nine different diets, displayed in Table 1. The non-fat milk was prepared from dry milk according to the package instructions. Beef liver was free from hormones or antibiotics and purchased at a local grocery store. 100% pure honey was also purchased from a local grocery store and followed FDA's requirements for consumable honey. The measured amount of honey:water solution was applied to half of the tri-fold paper towel and then placed on half of the cell-culture dish to ensure contamination did not occur. For the ad libitum diets, the paper towel was completely soaked in solution and then placed in the dish. The honey:water solution was made fresh every other week and kept refrigerated to prevent bacterial colonization. Cages that received 1:1 honey:water solution received fresh solutions every other day and the paper towel removed at time of replacement. Cages that received milk and/or liver would receive an aliquoted amount of either supplement on days opposite of when the honey:water solution was replaced.

Each day, the cages were examined for deaths, any deaths were recorded according to sex. Every other day, fresh liver was placed in the cages, and the liver already present in the cage was checked for eggs. If eggs were present, they were counted, photographed for analysis, and documented.

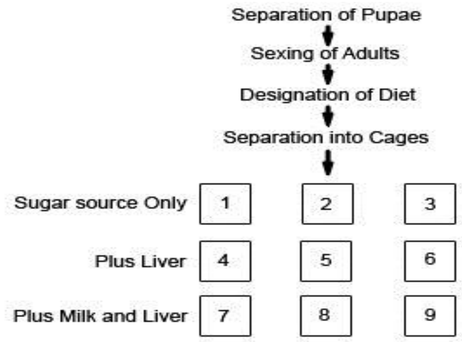

Fig. 1. Flow chart showing details of the experimental design

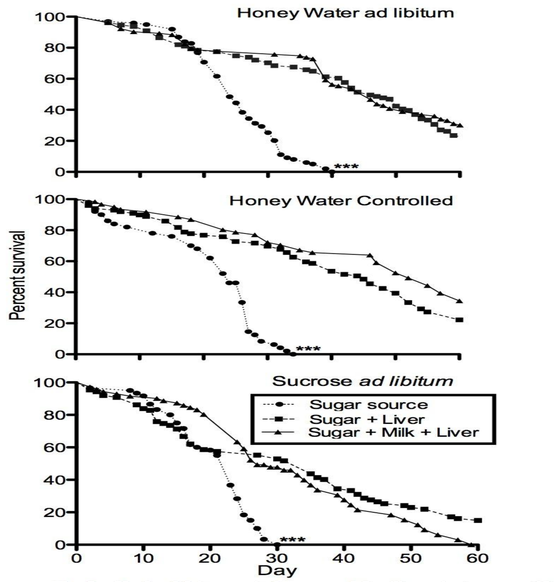

Fig. 2. Kaplan-Meier survival curves of *Lucilia sericata* over a 60 day period when fed one of three sugar sources (Honey Water *ad libitum*, Honey Water Controlled, or Sucrose *ad libitum*) with the addition of protein (Non-fat Milk and Liver, or Liver only). Significant differences between survival curves (Mantel-Cox log-rank test) are indicated by stars (*).

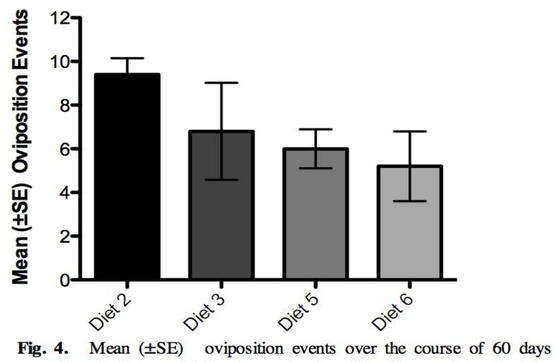

Fig. 4. Mean (±SE) oviposition events over the course of 60 days according to diet, combining two cohorts. Significance determined by Bonferroni post-tests after analysis by one-way ANOVA.

Results

Regardless of the sugar source, the survivorship of flies was influenced with the presence of a protein source in the diet. The flies fed on sugar alone died by Day 40, but a diet with protein extended the lives of the flies between 20 days to 70 days (Fig. 1). Flies fed sucrose only died the earliest in the study at 27 days. The flies that were fed a controlled amount of Honey Water plus milk and liver lived the longest at 112 days.

With cohorts combined, the number of times oviposition events occurred was not significantly different between diets, whereas oviposition events were significantly different between diets in the second cohort. Those flies fed milk and liver experienced more oviposition events than diets supplemented with liver only. Regardless of the protein source, flies fed Honey Water diets laid an average of three times more eggs than those given Sucrose over the course of 60 days. Most eggs laid per cage seemed to occur when there was between 4 and 14 females present, with egg production peaking with 10 females. Finally, the correlation between number of eggs oviposited and the number of females is significant only for flies fed Sucrose and liver.

Discussion and Conclusions

From the data presented, a diet of *ad libitum* Honey Water and bovine liver produces longevity and increased fecundity in adult *Lucilia sericata* blow flies. This experiments support the claim in that a protein source is vital for extending the lifespan of both the male and female blow fly. From our data, we have shown that with the proper sugar source and protein supplementation, flies can be expected to live well beyond 60 days (Fig 2). When liver, serving as the protein source, is given to the flies between post-ecclosion Day 10 and 20, a positive effect to survivorship occurs.

Comparing the survival curves of the Honey Water *ad libitum* and the Honey Water Controlled diets, there was no significant difference (Fig 2). However, there was a significant difference in the survivorship of flies fed Honey Water and those fed Sucrose. We hypothesize that a diet subsisting mainly of dry sucrose has the potential to dehydrate the flies, thereby possibly contributing to their early expiration. In comparing the number of oviposition events, both Honey Water diets outperformed the Sucrose diets, and sucrose supplemented with both milk and liver had the least oviposition events during the time period.

Both Honey Water treatments followed the same basic pattern in number of eggs produced, while there was a large discrepancy between those and the Sucrose diets. Those diets supplemented with both milk and liver did show significant trends between egg number and area. The trend was inversely proportional – as the egg number increased, the size of the egg decreased. This suggests that exposure to milk has a twofold effect: it allows earlier production of eggs and allows the production of larger eggs, and thus a higher chance of larvae hatching.

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Acknowledgements

We would like to thank Marie McDaniel for her contribution to this project. *This work has been supported in part by the University of Dayton Office of Graduate, Professional & Continuing Education through the Graduate Student Summer Fellowship Program.*