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Density and Feeding Related Mortality of *Musca domestica* (Lin.) Larvae (Diptera: Muscidae)

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Abstract Effects of varying densities of larvae and feed with an amino acid lysine (1.5g) additive on the mortality of larvae and growth of pupae of *Musca domestica* (L.) were determined. Larvae were initially kept at density of 3 larvae per group in a beaker and fed with 40 per cent milk (w/v) concentration. Both the density and feeding concentrations were increased progressively. Mortality of the larvae was zero at 3 larvae per group but the mean axial ratio of puparia was observed to be 13.75±0.33. As the number of larvae increased subsequently from 3 to 6, 12, 24, 42 and 74 per each group, varying mortality values and marked reduction in axial ratios of the puparia were observed. Increasing mortality rate was also observed with the constant number of larvae kept at 24 per group at different feeding regimes in some cases. Lysine additive however, seemed to influence the reduction of mortality rate but only slightly improved mean axial ratios of puparia. This suggested that factors other than food also affected survival of larvae and growth of pupae in crowding conditions.

Keywords Crowding, Feeding regimes, Larvae, Mortality, *Musca domestica*

1. Introduction

Musca domestica (L.) commonly known as housefly is widely distributed and familiar to many as domestic pest, nuisance fly and vector of variety of pathogenic organisms. Its serious insect not only for their annoyance factor but also for being major vectors of several disease- inducing agents [1]. Similarly, its habit of visiting garbage, excrement and human food all in one short flight makes it capable of transferring organisms that cause diarrhea, dysentery, typhoid, cholera and other diseases to human [2]. Expanding of the fly food range to include human garbage and moving indoors makes it to become a cosmopolitan pest and found wherever humans have settled [3,4]. *M. domestica* represents pest of major economic significance and pollution of animal products, poultry, livestock and the transfer of the wide range of animal pathogens [5]. The continued success of *M. domestica* in different parts of the world can be explained by at least two important factors, a high growth rate and short life cycle [6]. In fact, housefly represents the ultimate adaptation to the human environment by an insect [7].

The fly is a holometabolous with distinct egg, larva or maggot, pupa and adult stages. The whitish eggs each measured 1-2mm in length are laid singly and directly on acceptable substrate but pile up in small masses [8]. A good breeding medium is animal or poultry manure [9]. Up to 500 eggs can be laid per female in several batches of 75-150 over a three to four day period [10]. Number of eggs produced however, is a function of female size, which is principally a result of larval nutrition [11,12]. Normally the egg laying females are attracted to warm and moist (40-70% water) organic substrates that may provide adequate nourishment for the hatched larvae [7]. Maximum egg production occurs at intermediate temperature, 25-30℃ [13,14]. The first instar larva (3-9mm long), a typical creamy whitish in colour, cylindrical but tapering toward the head [15,16], hatched in about 8 hours or up to 3 days depending on temperature [7,13]. Larvae burrow into the food material to feed, but complete the three instars (3^{rd} instar larvae, 7-12mm long) and pupate in a week or less [17,18]. The optimum temperature for larval development is 35 to 38℃, though larval survival rate is highest at 17-32℃ [19,20]. Pupa is dark-brown 8mm long and under warm conditions last 4-6 days [7,16]. The emerging adult fly, 6-9mm in length [7] escapes from the pupa case through the use of an alternately swelling and shrinking sac called the ptilinum on the front of its head, which it uses like pneumatic hammer to break through the case [8,12] with the female usually larger than the male [16]. Warm summer conditions are generally optimum for the development of the housefly and it can complete its life cycle in as little as seven days [14]. As many as 10-12 generations may occur annually in temperate regions, while more than 20 generations may occur in subtropical and tropical regions [21,22]. Previous work done on housefly have indicated that larval crowding affect

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pre-imaginal mortality [23], adult size proportional to life span [24] and that several factors like size and aggressiveness determined success in competition for food [25].

2. Objectives

This paper determines the effect of three variables; larval crowding, feeding regimes and lysine additive on the mortality of housefly larvae and size of the puparia.

3. Materials and Methods

3.1. Collection of the Flies

Adult houseflies, *M. domestica* were collected from insect swarm in Sokoto main abattoir using insect sweep net. The technique used was by throwing the net at angle of 45° to the horizontal and then rose to about 90°; in this a lot of the flies were caught. This was immediately followed by squeezing the top side of the net with hand to disallow any fly escape. The adults were brought to the laboratory where both sexes were sorted and identified through taxonomic procedure [26].

3.2. Breeding Procedure

For the rearing purpose, adult flies were transferred into a population cage sized 30 x 30 x 30cm covered with mosquito net, having one side provided with a sleeve to allow for easy access to the inside through which food container could pass in or out of the cage. Floor of the cage was sprayed with green manure to enhanced oviposition. The flies in the cage were fed with 40% (w/v) of NIDO milk solution soaked in 30g of cotton wool, since the adults would require food (protein) before copulation can take place [22]. Eggs laid by the female flies in Petri dishes and on cotton wool already placed in the cage were collected and placed in a beaker (100mL capacity) containing three pieces of 20g cotton wool moist with 40% (w/v) of NIDO milk solution. The beaker was covered with muslin cloth to keep away other insects from entering. After every 24hrs, the cotton wool was re-moist again with the same milk solution. Hatched larvae obtained in the beaker were allowed to pupate and the resulting pupae were placed into a similar population cage. Within three weeks of this procedure, large number of adults was obtained. The purpose was to procure a third generation flies from which eggs were removed and reared for the experiment.

3.3. Experiment Conducted

From the third generation flies maintained as stock, variable number of individuals per group of larvae (3, 6, 12, 24, 42 and 74) was sampled using camel hair brush and then placed in separate but similar beakers containing cotton wool soaked in 40% (w/v) milk solution to serve as larval food. Six concentrations of the milk were prepared (15, 20, 40, 60, 80 and 100% w/v) and from each also, cotton wool was soaked and placed in 100mL beaker to feed 24 larvae within. In another set up, while maintaining different concentrations of milk feed and density of larvae at 24 per group, additional cotton wool soaked in 1.5g amino acid lysine solution was also placed to feed the larvae on each concentration. Observations were made daily for larval mortality and all the larvae considered dead were removed from the beaker and their number was counted. The axial ratio of puparia (size of the pupa) was measured in millimeters with venire calliper (Mutoyo: Japan) upon pupation before eclusion. All experiments were replicated three times.

3.4. Data Analysis

Larval mortality was expressed in per cent as the difference in the number of dead larvae and pupae to the initial number of larvae introduced [27]. Analysis of Variance was used to calculate the mean size of pupae and expressed as the mean axial ratio.

4. Results and Discussion

Crowding effect on the mortality of *M. domestica* larvae is presented in Table 1. There was no larval mortality observed in less crowding condition of three larvae per beaker, while the mean axial ratio of the resulting pupae was observed to be 13.75±0.33. When the density of larvae was increased twice, a record of 16.7% mortality was obtained indicating that the effect of crowding was slowly building but the mean axial ratio of pupae had only changed slightly (13.46±0.53) from the previously observed mean when larval crowding was as least. This observation could not be rationally attributed to inadequate space or food since both were available in this case. However, some authors have proposed possible factors for relatively high mortality at very low densities in tadpoles of *Rana pipiens* as polluting effect of food excess caused by increase in dissolved oxygen or increase in toxins [28]. It was confirmed that during larval stage of *M. domestica* there was elimination of ammonia which is toxic and also oxygen consumption decreases while carbon dioxide production increases [29]. Larval mortality steadily increased with every rise in crowding, so also the reduction in the pupal mean axial ratio with the highest reduction (11.85±1.20) observed in highest larval density (74 per beaker). Similar observations were made previously when working with *Drosophila melanogaster* [15, 25] and with *M. domestica* [30].

Percent mortality of the larvae had changed when the larvae were fed with varying concentrations of milk feed as shown in Table 2. The highest percentage mortalities were 29.2% and 29.0% respectively observed for 15% and 20% milk (w/v) concentrations. However, the least percentage mortality was recorded for 60% milk (w/v) concentration (15.6%). The remaining concentrations of milk feed were observed for relatively similar percentages of larval mortality. High larval mortality at densities of 42 and 74 larvae per group suggest that the effective density of the larvae became more operational. This could apparently lead to the larval competition for food which could make them aggressive, with the less aggressive ones being eliminated. Other reasons might include behavioral, social and physiological stress acting through mechanoreceptors [31] encountered during competition not only for food but also for space as well. Similarly, limiting of certain nutrients in food due to competition can produce an unstable physiological state described as shock disease [32], the result of which is usually death. Most of the negative effects of high density occurred during the final larval instar, probably resulting from food depletion. Another possibility is larval mortality through mechanical injury. A report has shown that *M. domestica* larvae possess hook-like structures used for tearing food and for locomotion [22,33]. Thus, larvae might have inflicted fatal wounds on one another or contracted deadly infections through the wounds. The observed advantage of this phenomenon is increased total survival rate and larger flies through reduced density during the growth phase of the remaining final larval instar [8,34]. Cause of larval mortality might also be partly nutritional because when milk concentrations were low (15% and 20%) morality was observed to be high which presumably indicate inadequate food supply. Early insufficient nutrition may result in poor medium conditioning and death of larvae [30,36]. Turnaround in the level of mortality was the case upon increased milk concentrations above 20% (w/v). It appears that the larvae preferred feeding more within 60% milk concentration where relatively lesser mortality levels had occurred. This might not be unconnected with the adult females' preference of 40-70% moisture content of substrate for oviposition [7] and possible easy feeding of hatched larvae.

Table 1. Density related mortality of *M. domestica* larvae fed with 40% milk feed (w/v)

Larval density per beaker	Mortality (%)	Mean axial ratio (mm) of pupae ± SE	Range
3	0.0	13.75±0.33	13.42-14.08
6	16.7	13.46±0.53	12.13-15.04
12	18.3	12.05±1.02	12.03±14.07
24	18.7	12.00±0.65	11.87-13.17
42	35.7	11.99±3.12	11.18-13.22
74	37.8	11.85±1.20	10.65-13.05

Mean value represents three replicates

Similarly, the mean axial ratios of pupae were at par in most cases of feed concentrations, but the observed initial reduction of the axial ratios in conditions of higher larval density may be explained also in terms of effective density of the population due to stress. This has been earlier on reported, that mechano pressure act through mechano-receptors inhibiting the production of growth hormones [16,35]. However, some other workers could not establish the co-relation between crowding and size of puparia [10]. Table 3 depicts the per cent larval mortality in milk feed concentrations, each added with 1.5g of amino acid lysine. Though, in all concentrations the number of mortality had reduced, the effect of lysine probably in this regard was more pronounced in 60% milk (w/v) feed concentration (6.5%). When the concentration was raised from 60% and above, larval mortality has also increased to 10.7% and 15.1% respectively in 80% and 100% feed concentrations. However, the means of axial ratios of puparia remained without pronounced variations among all pupae. Thus, the expected increase in the size of pupae due to achieve through lysine additive has not been achieved, rather, the size of puparia remained at par. It was possible that the larvae which developed into pupae have not received the optimal balanced diet required to permit for full potential size (14mm) of pupae. Larval diet normally has an impact on the subsequent pupa size [37,38]. A previous study indicated that the amino acid profile of *M. domestica* larva is well balanced with no limiting amino acids, but modestly lower levels of leucine and lysine were observed [39]. It was in this respect that lysine as an essential amino acid was added to raise the nutritional value of the feed in order to promote growth of the insect. Understanding the relationship between variables studied in the current study can help scholars in maintenance of laboratory culture of *M. domestica*. Similarly, with the urge for protein supplementation that can be provided by insects in poultry feed, *M. domestica* can be a good candidate. More systematic work in the 1970s and 80s highlighted some of the distinct potentials of using specific species of insect larvae in aquaculture and livestock production [40].

Table 2. Milk feed mortality related of *M. domestica* larvae maintained at density of 24 larvae per group

Milk feed % (w/v)	Mortality (%)	Mean axial ratio (mm) of pupae ± SE	Range
15	29.2	12.48 ± 0.67	11.84-13.12
20	29.0	12.48±0.65	11.80-13.06
40	16.4	12.53±0.63	11.91±13.15
60	15.6	12.57±0.53	11.88-13.16
80	16.0	12.55±0.61	11.99-13.05
100	16.8	12.51±0.64	11.88-13.14

Mean value represents three replicates

Table 3. Mean axial ratio of *M. domestica* larvae fed with 1.5g lysine additive in milk feed at density of 24 larvae per group

Milk feed% (w/v) plus 1.5g lysine	Mortality (%)	Mean axial ratio (mm) of pupae ± SE	Range
15	26.2	12.50±0.60	11.86-13.05
20	25.8	12.50±0.73	11.89-13.15
40	11.6	12.53±0.62	11.87±13.16
60	6.2	12.54±0.55	11.99-13.09
80	10.7	12.53±0.58	11.95-13.11
100	15.1	12.53±0.66	11.87-13.19

Mean value represents three replicates

5. Conclusions

This study has shown that, crowding of *M. domestica* larvae was indirectly proportional to larval mortality. That the larvae have shown a particular preference for milk feed concentration but the amino acid lysine additive in feed could not achieve marked increase in the axial ratio of puparia. Some other factors not exploited presently might also be responsible for high larval mortality.

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